EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ AND NORGESTOMET ON ESTRUS SYNCHRONIZATION AND FERTILITY IN POSTPARTUM BEEF COWS

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ABSTRACT

Experiments were carried out aiming to evaluate stage of estrous cycle on fertility of a synchronized estrus and to determine the minimum effective level of norgestomet delivered from silicone implants capable to suppress estrus for a 16 day period of resynchronization. Postpartum suckled beef cows (n=270) in two locations were randomly allotted in two treatments with (n=134) or without (n=136) a luteolytic dose of $PGF_{2\alpha}$, dinoprost tromethamine five days prior to the synchronization of estrus with Syncro-Mate B® (SMB). SMB consisted of an injection of 5 mg estradiol valerate and 3 mg norgestomet and the insertion of a 6 mg norgestomet implant. The implant was removed 9 days later and the cows were artificially inseminated 48 after implant withdrawal. The pretreatment with $PGF_{2\alpha}$ reduced ($p<0.05$) calving rate to the timed artificial insemination when days postpartum was included in the statistical model as a covariate. Significant differences ($p<0.05$) in fertility were found according to stage of estrous cycle at the initiation of SMB, regardless of treatment. Calving rate was significantly higher ($p<.05$) when cows were synchronized with Syncro-Mate B during the second half of the cycle (35/79 = 44.3%) compared to cows either in anestrous (19/78 = 24.3%) or in the first half of the cycle (22/107 = 20.6%). The daily release of 136 μg of norgestomet effectively suppressed estrus in beef cattle. An additional trial was conducted to evaluate silicone implants impregnated either with 6 mg or 8 mg of norgestomet and inserted five days after an initial service for the resynchronization of the

return estrus in cows previously treated with SMB. There was no effect ($p>.75$) of type of second implant on the calving rate to the return service. Overall combined, i.e. first added to second artificial insemination, calving rate reached 57.1% and breakthrough estrus was observed in 17.6% cows implanted with 6 mg norgestomet capsule. In conclusion, silicone implants impregnated with 8 mg of norgestomet allowed for a second service at predetermined time and increased the number of calves born to artificial insemination.

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CHAPTER I.

LITERATURE REVIEW

In the last few years there has been a steadily decrease in per capita beef consumption (Savell et al., 1989). However, the United States still remains as the world's largest consumer of beef. In addition to supplying its own market, the American beef industry provides a surplus of commodities for exportation. In 1992 U.S. beef and veal exports generated more than 2 billion U.S. dollars, not including 321,790 head of cattle exported in that year (World's Livestock Situation, 1993). However, population growth and its inherent demand for animal products will increase at an even faster pace (Cartwright et al., 1980) and is likely to offset modest growth in beef production.

Reproductive efficiency is recognized to be the single most important limiting factor in maximizing beef productivity and profitability (Neumann and Lusby, 1986). The reduction of the interval from parturition to conception increases reproductive efficiency in farm animals (Wettemann, 1980). A 21 day delay in rebreeding can result in an estimated loss of 28 to 52 U.S. dollars the following year (Favero, 1992). Therefore, rebreeding of postpartum cows at earliest opportunity improves reproductive rates of cows and maximizes the profits to be obtained from them. Enhancements on the reproductive rates should be accompanied by improvements of the genetic merit of the herd. Cartwright et al. (1980) stated that genetic improvement is a continuous process that should involve a strategy for coping with goals that change with

time. A remarkable example is given by Savell et al. (1989) who emphasized that beef industry must be aware of the changes in consumer's behavior towards red meats because consumers have become increasingly conscious of their diet and the effect it may have on their health. This represents an additional challenge to beef producers, who need to quickly adapt their production systems and types of animal to fit the consumer's demand for leaner and still tasty cuts of meats. Use of superior genetics through the use of artificial insemination (Mikeska and Williams, 1988; Odde, 1990; Favero, 1992; Mutiga et al., 1993), embryo transfer (Favero, 1992; Mutiga et al., 1993), and embryo cloning (Favero, 1992) can accomplish such a task. However, the control of reproductive events in a beef cattle operation is complicated by the unique management on each farm. Presently, less than 5% of the national beef cow herd is artificially inseminated annually (Odde, 1990). Labor required to accurately detect estrus (Valle, 1986; Odde, 1990; Favero, 1992) and the proper timing of insemination relative to the time of ovulation (Larson end Ball, 1992; Thibodeaux et al., 1992) are considered to be major factors limiting the wide-spread use of artificial insemination among commercial beef producers.

The manipulation of the estrous cycle or induction of estrus to bring a large percentage of a group of females into heat at a predetermined time would surely facilitate the use of the artificial insemination (Mikeska and Williams, 1988; Odde, 1990; Mutiga et al., 1993) by reducing the labor involved (Favero, 1992; Gaines et al., 1993). This manipulation is known as estrus

synchronization (Whittier et al., 1986; Odde, 1990). In addition to its effect on the increasing of the number of cows artificially inseminated on a per year basis, estrus synchronization might also enhance reproductive performance by allowing for a shortened breeding and calving season (Wiltbank, 1974; Odde, 1990; Tibary et al., 1992), and increase the weight of the weaned calves (Odde, 1990; Tibary et al., 1992; Gaines et al., 1993).

In cyclic cows, the time of estrus is controlled by secretion of progesterone from the corpus luteum. Progesterone exerts a negative feedback on luteinizing hormone (LH) secretion so that the endocrine events that lead to the maturation of preovulatory follicles and their subsequent ovulation are inhibited until progesterone declines at the time of corpus luteum regression. Thus, synchronization of estrus and ovulation is accomplished by controlling the life span of the corpus luteum (Hansel and Convey, 1983).

Several methods to control estrus and induce ovulation in cattle have been developed and comprehensive reviews are available (Cooper, 1978; Odde, 1990; Larson and Ball, 1992). Odde (1990) grouped the methods to synchronize estrus according to the type of compounds employed in the protocols and they are as follows: progestogen-based; combinations of progestogen and estrogen-based; prostaglandin $F_{2\alpha}$ or its analogues-based; combinations of progestogen and $PGF_{2\alpha}$-based programs.

The progestogen-based programs evolved from the results described by Christian and Casida in 1948 (Cooper, 1978), who

successfully synchronized estrus in cattle using daily injections of progesterone. Since then, a variety of synthetic progestational agents administered by various routes and at varying doses have been reported to effectively suppress estrus in cows (Cooper, 1978; Odde, 1990). It has been established that long term (18 to 21 days) administration of progestogens reduces the fertility of this synchronized estrus (Hansel et al., 1961). Stimulation of follicular atresia, reduction of sperm transport, altered fertilization rate and retarded embryo cleavage rate are some possible effects of prolonged exposure to progestogens (Odde, 1990). The use of long term oral administration of progestogens has been shown to keep feedlot heifers from estrus and thus improve weight gain and profitability (Neumann and Lusby, 1986; Favero, 1992).

The progestogen-estrogen combination programs shortened the exposure to progestogens because of the incorporation of a luteolytic agent, estrogen, into the protocol. The luteolytic properties of estrogens were inferred from the fact that at high dosages, estrogens caused a great loss of weight in corpora lutea in 80 to 90% of treated animals within 8 days and a decrease in progesterone concentration in corpora lutea as well (Denamur, 1968). The combination of norgestomet, a potent progestogen, and estradiol valerate was devised to synchronize estrus in cattle (Thimmonier et al, 1975) and perfected by Wiltbank and Gonzalez-Padilla (1975). This protocol became commercially available under the name of Syncro-Mate B and has been efficacious in inducing

estrus and ovulation in both anestrous and cyclic cows (Miksch et al., 1978).

However, conception rates at the Syncro-Mate B synchronized estrus have been variable (Odde, 1990; Larson and Ball, 1992) and pregnancy rates in postpartum beef cows either after a single timed-artificial insemination or over a five day period ranged from 28% (Kiser et al., 1980) to 64% (Miksch et al., 1978). The major sources for variability already recognized are: the nutritional status of the herd and the body condition of the animals (Odde, 1990), interval postpartum (Odde,1990), skillfulness of inseminators (Washburn and Dailey, 1987; Richards et al., 1990; Larson and Ball, 1992), asynchronism between the onset of estrus and the LH surge (Mikeska and Williams, 1988), stage of estrous cycle at initiation of treatment (Spitzer et al., 1978; Whittier et al., 1986; Brink and Kiracofe, 1988; Richards et al., 1990; Fanning et al., 1992; Larson and Ball, 1992; Bo et al., 1993), proportion of animals cycling before treatment (Richards et al., 1990; Larson and Ball, 1992), slow or incomplete luteolysis by estradiol valerate (Whittier et al., 1986; Mikeska and Williams, 1988; Larson and Ball, 1992; Twagiramungu et al., 1992; Bo et al., 1993), luteal dysfunction after treatment (Favero et al., 1988), unsuccessful establishment of pregnancy with associated embryo mortality (Cartwright et al., 1980), altered follicular dynamics (Bo et al., 1993), suckling and calf removal (Valle, 1986), and gonadotropin support therapy (Valle, 1986; Favero, 1992).

The $PGF_{2\alpha}$ or its analogue-based programs are used to synchronize estrus in cattle because of the ability of these compounds to cause luteolysis (Melampy and Anderson, 1968). However, $PGF_{2\alpha}$ and its analogues are ineffective in causing luteolysis in the early stages (prior to day 6) of the estrous cycles (Hafs et al., 1974; Odde, 1990). Therefore, two injections given 10 to 12 days apart are required to place most of animals at a stage of the estrous cycles when they respond with luteolysis, follicular growth, estrus and ovulation (Cooper, 1978). This procedure will not bring acyclic cows into estrus (Brown et al., 1988). The response of cyclic cows also depends upon the stage of the estrous cycle at the time of $PGF_{2\alpha}$ injections (Odde, 1990). It has been demonstrated that two timed artificial inseminations are needed to ensure acceptable levels of pregnancy (Hafs et al., 1974; Cooper, 1978), which varied from 23% up to 46% (Lauderdale et al., 1980) in lactating beef cows.

Programs combining progestogens and prostaglandins have been developed (Odde, 1990), where $PGF_{2\alpha}$ or one of its analogues is administered near to the end of implant period and corpus luteum demise is ensured (Richards et al., 1990; Twagiramungu et al., 1992). Estrus synchrony reaches nearly 100% (Twagiramungu et al., 1992) and conception rates achieved are equivalent to those obtained through the Syncro-Mate B program in beef cattle (Odde, 1990; Twagiramungu et al., 1992).

A tightly synchronized estrus in a group of cows allows a single timed artificial insemination with fertility level

comparable to that observed in a natural estrous cycle. Unfortunately, the available protocols for estrus synchronization do not achieve the desired degree of synchrony because estrus is displayed over a period of five days or longer. This situation led producers to use estrus synchronization coupled to estrus detection for a shortened period. Such a procedure adds labor, costs and makes timed breeding unfeasible. Recently, attempts have been made to allow a second timed artificial insemination (Stevenson and Mee, 1991; Favero, 1992; Sinclair et al., 1992; Domatob, 1993), which would favor the utilization of artificial insemination in a larger number of cows and result in a greater number of calves born earlier in the calving season.

The effects of the synchronization of estrus upon the subsequent estrous cycles are related to hormonal imbalances after synchronizing treatments. Voss and Holtz (1985) reported that 51% and 53% of cows treated with $PGF_{2\alpha}$ and Syncro-Mate B respectively, showed abnormal estrous cycle length after treatments, compared to 12% in the untreated cows. In addition, Morrel et al. (1991) observed a reduction in conception rates after artificial insemination of heifers which were repeatedly treated with cloprostenol ($PGF_{2\alpha}$ analogue) to synchronize estrus. The degree of reduction was related to the number of synchronization treatments the animals had received. On the other hand, Hafs et al. (1974) did not find residual effects of estrus synchronization upon the hormonal profiles of the subsequent estrous cycle.

The reproductive potential of farm animals could be better explored if more knowledge about estrus, ovulation and fertilization were available. Actually, Cartwright et al. (1980) emphasized the need to amplifying research efforts on these areas of animal reproduction in order to cope with the urgent demand for animal products in the 21st century.

Cattle producers base their evaluation of estrus synchronization programs upon reproductive results and other non-biological concerns such as safety of handling, ease of execution and economics (Cooper, 1978; Larson and Ball, 1992; Sleening, 1992) and the relative importance placed on each factor varies greatly for different producers (Odde, 1990). Therefore, estrus synchronization protocols should be designed to optimize the biological response without compromising the economic return.

CHAPTER II.

EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$-PRETREATMENT ON THE CALVING RATE OF POSTPARTUM BEEF COWS SUBMITTED TO ESTRUS SYNCHRONIZATION WITH AN ESTROGEN-NORGESTOMET COMBINATION

SUMMARY

Two-hundred and seventy cows at two locations averaging 62.78 ± 17.51 days postpartum were synchronized into estrus with a 6 mg silicone norgestomet implant subcutaneously inserted in the ear at the same time as an intramuscular injection of 3 mg norgestomet and 5 mg estradiol valerate was given. Cows were bred by artificial insemination approximately 48 hours after implant removal. One-hundred and thirty-four cows received 25 mg of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as dinoprost tromethamine five days before estrus synchronization. Overall implant loss rate was 1.5% (4/270). 70.4% of cows were cycling at implant insertion and the degree of cyclicity was related to days postpartum. On the average, cyclic cows were at 67.65 ± 16.12 days and anestrous cows were at 51.50 ± 16.44 days postpartum at the initiation of treatment. Luteolysis took place in 65.5% of $PGF_{2\alpha}$-treated cows which were bearing corpora lutea at the time of injection and spontaneous regression occurred in 40.7% of control cows ($p<.05$). Differences between treatments in calving rate were significant ($p<.05$) when days postpartum was included as covariate in the model and calving rates were: 33.1% for control and 23.7% for $PGF_{2\alpha}$-treated. The effect of days postpartum was significant ($p<.05$) but no binary interactions

between factors studied (treatment, location, cyclicity, stage of estrous cycle, and progesterone concentration at the time of insertion of implant) were significant (p>.40). Induced regression of corpus luteum by means of $PGF_{2\alpha}$ displaced cows at luteal stage (n=38) towards metestrus at the time of Syncro-Mate B treatment and fertility was significantly(p<.05) depressed (7.9%) whereas spontaneous regression did not affect calving rate (50.0%). In conclusion, administration of $PGF_{2\alpha}$ is not recommended five days before estrus synchronization with a norgestomet-estradiol valerate combination.

INTRODUCTION

Postpartum anestrus is a period of reproductive quiescence after the parturition. It is associated with a variable endocrine profile (Erb et al., 1971; Wagner and Oxenreider, 1971; Blasco and Revilla, 1992), uterine involution (Kiracofe, 1980) and characterized by a lack of follicular maturation and luteal development (Wettemann, 1980). The postpartum interval is the period of time from parturition until the first postpartum estrus (Dunn and Kaltenbach, 1980) and its length varies greatly in beef cattle, where intervals as short as 15 days and longer than 100 days have been reported (Dunn and Kaltenbach, 1980; Wettemann, 1980). The major factors lengthening the postpartum interval are: puerperal abnormalities coupled with retarded uterine involution (Kiracofe, 1980), suckling and low dietary energy intake (Wagner and Oxenreider, 1971) and body condition at both pre- and post-

calving (Dunn and Kaltenbach, 1980). In addition, the first ovulation in beef cattle frequently is not accompanied by behavioral estrus and the subsequent luteal phase is shorter than normal (Wettemann, 1980). Attempts have been made in order to hasten uterine involution (López-Gatius and Camón-Urgel, 1989; Archbald et al., 1990; Glanvill and Dobson, 1991; Morton et al., 1992) with $PGF_{2\alpha}$ and to shorten interval postpartum (Valle, 1986; Favero, 1992) with gonadotropin release hormone (GnRH) associated with or without short-term calf removal.

The wide variety of beef production systems and distinct environmental conditions in the United States obligates cattlemen to control reproductive rate according to biological concerns, technological advances, and changes in economic circumstances. However, a 365-day calving interval is generally considered to be optimum for most American beef systems (Casida, 1971). Therefore, a cow must conceive by 80 days postpartum to show the desired interval between successive parturitions (Casida, 1971; Dunn and Kaltenbach, 1980).

An ideal program to synchronize estrus in postpartum suckled beef cows would have to fulfill some requisites, such as being capable of producing normal ovarian activity in cows still in anestrus; to provide a tight degree of synchrony of estrus and ovulation allowing for a single-timed artificial insemination; and to achieve high fertility after the appointed artificial insemination. This should make it cost-effective and to ensure adequate conditions for early embryonic development by preventing

short-lived corpus luteum. The complete fulfillment of these requirements would accomplish the main goals of estrus synchronization, which are the facilitation of the use of superior genetics through the artificial insemination and the enhancement of the reproductive efficiency by shortening postpartum interval, breeding and calving season (Odde, 1990). As a matter of fact, most of estrus synchronization protocols aim to regulate reproductive cycles by means of a tight control of corpus luteum regression. The luteolytic properties of estrogens (Wiltbank et al., 1961) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its synthetic analogues (Melampy and Anderson, 1968) have been demonstrated to cause a demise of the corpus luteum in cattle leading to a sharp fall in progesterone secretion (Denamur, 1968; Hafs et al., 1974; Wettemann, 1980; Mutiga et al., 1993), an increase in estradiol (Hafs et al., 1974) and ultimately a surge of LH associated with estrus and ovulation (Hafs et al., 1974; Archbald et al., 1990; Mutiga et al., 1993). Therefore, effectiveness of these compounds to induce ovulation in non-pregnant cows requires them to be cycling and at luteal phase of the estrous cycle (Hafs et al., 1974; Logue et al., 1991). In addition to this limitation, estradiol valerate does not reliably cause luteal regression (Twagiramungu et al., 1992; Bo et al., 1993) even when used in association with progestogen (Brink and Kiracofe, 1988; Pratt et al., 1991; Fanning et al., 1992). Likewise, the luteolytic action of $PGF_{2\alpha}$ and its analogues depends on the status of the corpus luteum preceding the treatment (Odde, 1990; Larson and Ball, 1992)

and there is an individual variability in response among cows (Garcia-Winder and Gallegos-Sanchez, 1991).

The use of a combination of a 6 mg norgestomet implant left in situ for 9 days associated with an intramuscular injection of 3 mg norgestomet and 5 mg estradiol valerate at implant insertion has been capable of effectively synchronizing estrus in beef cows (Cooper, 1978; Odde, 1990), inducing ovulation in both cyclic (Wishart and Young, 1974) and anestrous postpartum beef cows (Miksch et al., 1978), and preventing the early demise of the first postpartum corpus luteum (Kesler et al., 1980; Troxel and Kesler, 1984; Copelin et al., 1988; Richards et al., 1988; Mee et al., 1991; Coy and Garcia-Winder, 1991). The mechanics of treatment relies upon the fact that the norgestomet implant serves as an estrus suppressor and the injection of norgestomet and estradiol valerate is assumed to inhibit corpus luteum formation or initiate corpus luteum regression (Pratt et al., 1991). This protocol is marketed under the name of Syncro-Mate B® (CEVA laboratories, Overland Park, KS). Significant differences in conception rates across trials have been consistently reported after Syncro-Mate B treatment (Odde, 1990). It was established that stage of estrous cycle at initiation of progestogen treatment affects the duration of exposure to progestogens (endogenous progesterone plus exogenous progestogen) and fertility (Larson and Ball, 1992). Ovulation was suppressed for a longer period when progestogen treatment begun during the 11 to 22nd day of estrous cycle of cows and fertility to the fixed-time artificial insemination was reduced (Gyawu et al.,

1991). Kesler and Favero (1993) also observed that Syncro-Mate B treatment hastened luteal regression when administered prior to day 11 of the estrous cycle and as stage of estrous cycle increased, the Syncro-Mate B-induced suppression on progesterone secretion decreased. These findings corroborate the results previously described by Brink and Kiracofe (1988) who verified higher conception rate in cows treated with Syncro-Mate B early (≤ 11 days) compared to cows treated late (> 11 days) in the estrous cycle (63 vs. 46%, respectively). On the other hand, Pratt et al. (1991) found that a high percentage (from 67 to 92%) of cows implanted early (< day 5) in their estrous cycle had functional corpora lutea at the time of implant removal, compared to less than 1% of cows treated on day 9. A failure of Syncro-Mate B program to either promote luteal regression or completely prevent luteal development when treatment initiates within metestrus has been suggested (Fanning et al., 1992; Burns et al., 1993).

The objective of the present study is to evaluate the effect of pretreatment with $PGF_{2\alpha}$ in postpartum beef cows submitted to a Syncro-Mate B program on calving rate. It is hypothesized that cyclic cows at luteal stages would undergo luteolysis, cyclic cows either at proestrus or metestrus will be at early stage (≤ day 11) of estrous cycle at initiation of treatment and the Syncro-Mate B treatment will be capable to induce ovulation in those cows still in anestrus.

MATERIAL AND METHODS

This experiment was conducted at two locations in the state of Illinois and took place in the spring of 1992. The experimental animals consisted of suckled postpartum Angus and mixed breed beef cows of different ages and parities. Seventy-two cows maintained at Urbana South farms, Urbana, at 57.42 ± 15.00 days postpartum and one-hundred and ninety-eight cows maintained at the Dixon Springs Agricultural Center (DSAC), Simpson, at 64.61 ± 19.20 days postpartum were randomly assigned within farm to treated or control groups. All cows were synchronized by means of an intramuscular injection of norgestomet (3.0 mg) and valerate estradiol (5.0 mg) in a sesame oil and benzyl alcohol (10%) carrier. At the same time a silastic implant containing 6.0 mg of norgestomet was subcutaneously inserted into the convex surface of the ear. Cows from the treated group (n=36 in Urbana and n=98 in Simpson) had received an intramuscular injection of 25 mg of a $PGF_{2\alpha}$, as dinoprost tromethamine, five days prior to the estrus synchronization procedure. Control group cows (n=36 in Urbana and n=100 in Simpson) did not receive any injection. The implants were removed after 9 days <u>in situ</u>. Approximately 48 hours after norgestomet implant withdrawal all cows were artificially inseminated.

Blood serum was collected from all cows at strategic dates (eleven days before, at the same time, and five days after) regarding to $PGF_{2\alpha}$ administration to determine both the estrous cyclicity status of cows prior to synchronization and the

efficiency of the prostaglandin as a luteolytic agent. Blood was collected from each cow via jugular venipuncture into 15 ml test tubes and immediately placed in ice until centrifugation at 1000 x g for 15 minutes at +4°C. All samples were centrifuged within 6 hours of collection. Serum samples were then individually stored in 4 ml vials and kept at -20°C (Wiseman et al., 1982) until assayed. Progesterone concentration was determined through a validated enzyme linked immunosorbant assay (ELISA; Kesler et al., 1990). Progesterone concentration of 1.5 ng/ml or above in any of the three samplings was considered to indicate the resumption of ovarian activity (Favero, 1992). A decrease in progesterone concentration from ≥ 1.50 ng/ml (Ghallab et al., 1984; Favero, 1992) to < 1.0 ng/ml between the day of $PGF_{2\alpha}$ administration and the day of the insertion of the norgestomet implant was taken to indicate that luteolysis had taken place (Wettemann, 1980; Glanvill and Dobson, 1991).

An additional investigation was conducted solely with cows from Urbana (n=72). The experiment aimed to evaluate if silicone implants impregnated with different amounts of norgestomet were efficacious to resynchronize the return to estrus in previously synchronized inseminated but non-pregnant postpartum beef cows, thus allowing for a second timed-artificial insemination. The complete methodology, results and implications of this appended trial are fully described in the Chapter IV.

Only calvings 283 ± 11 days from the date of the initial artificial insemination were considered to determine calving rates for treated and control groups.

The Chi-square test in 2x2 contingency tables was used to determine differences between treated and control cows to the following parameters: cyclicity status of cows at the initiation of the estrus synchronization procedure, and efficiency of induction of luteolysis by $PGF_{2\alpha}$. Effects of location, treatment, cyclicity status, estrous cycle stage, and interactions among them on calving rate were assessed through general linear models procedures for analysis of variances and covariances (Steel and Torrie, 1980). Student's t test was used to proceed comparisons between days postpartum of the categories of interest. All analyses were run using the SAS program (SAS User's guide, 1985).

RESULTS AND DISCUSSION

During the course of field trial, four cows lost their implants, two others were missing for a blood collection. Therefore, a total of six cows was discarded from the experiment, and data from these animals were not used to evaluate the outcome of treatments. All these cows were from Urbana, as a result the number of cows per group on which data were evaluated was as follows: Urbana-Control: n=33, Urbana-$PGF_{2\alpha}$ treated: n=33, DSAC-Control: n=100 and DSAC-$PGF_{2\alpha}$ treated: n=98.

The implant loss rate to Urbana cows was 5.5% (4 lost/72 implanted) compared to 0.0% (0 lost/198 implanted) of DSAC and the

overall loss rate became 1.5% (4 lost/ 270 implanted). These values are within the ranges reported for both norgestomet impregnated hydron implants (Spitzer et al., 1978; Kerr et al., 1991; Logue et al., 1991; Archbald et al., 1992; Broadbent et al., 1992) and norgestomet impregnated silicone implants (Favero et al., 1993; Hill et al., 1992; Domatob, 1993) which are respectively between 0 and 14.5%, and 0 to 10.0%. Probable causes for implant expulsion include local infection associated with active tissue rejection (Spitzer et al., 1978; Hill et al., 1992), misplacing of implants (Spitzer et al., 1978) and lack of dexterity of the technician who inserts the implants (Hill et al., 1992). All implants utilized in this trial were previously covered with broad spectrum antibiotics, which have increased retention rates to near 100.0% (Hill et al., 1992; Favero, 1992). Insertion of implants was performed by two different technicians using different applicators at two different locations and loss rate differed ($p<.01$) between locations. Therefore, it seems reasonable to narrow the causative factors for implant losses to field problems associated with insertion and not to related to the manufacturing of the silicone implant. This confirms the non-antigenic and non-irritating properties of silicone polymers (Dziuk and Cook, 1966; Sun et al., 1986).

Loss rate for norgestomet-hydron implants seems to increase proportionally with the number of females handled. Kerr et al. (1991) reported a loss rate of 2.3% (1/43), Sptizer et al. (1978) reported a loss rate of 5.0% (3/60) and Broadbent et al., (1992)

reported a loss rate of 14.5% (29/200). Domatob (1993) employed a matrix silastic implant and obtained loss rate of 6.4% (28/400). The overall loss rate herein reported is lower (1.5%) than those previous findings with hydron implants or silicone implants. This result associated with the findings of Domatob (1993), who reported an acceptable loss rate considering the sample size, makes possible to assume that silicone implants are more biocompatible than norgestomet-hydron implants.

A higher biocompatibility can enhance the retention rate for prolonged steroid release systems and prevents problems caused by the loss of implants, which may jeopardize the results of an implant-based estrus synchronization program. The consequences of a lost implant are not only linked to the asynchrony but also to the hormonal imbalance generated (Broadbent et al., 1992), which has carry-over effects on the physiology and length of the following estrous cycle (McCartney et al., 1990; Favero, 1992). Losses also happen with other types of prolonged steroid release protocols, such as PRID, and loss rate oscillates in the same range reported for norgestomet-hydron implants (Kerr et al., 1991). In this scenario, increases of the implant retention rates can positively contribute to the overall outcome of the procedures and allow higher fertility rates after implant-based estrus synchronization programs.

The presynchronization reproductive status of cows is shown in Table 2-1. Overall, there were significantly ($p<.05$) more cyclic cows (186/264 = 70.4%) than anestrous cows (78/264 = 29.5%) at the

initiation of the estrus synchronization procedure. This pattern was the same for both locations ($p>.05$).

Table 2-2 shows the interval between parturition and commencement of the estrus synchronization procedure. The random assignment proceeded at the beginning of the experiment provided homogeneous distribution, in such a way that control (63.92 ± 15.55 days postpartum) and $PGF_{2\alpha}$-treated (61.62 ± 18.06 days postpartum) cows had equivalent ($p>.05$) intervals from calving to synchronization.

As expected, cyclicity degree showed to be directly related with days postpartum, which were 51.50 ± 16.44 for anestrous cows and 67.65 ± 16.12 for cyclic cows. For the same reason more cows were cycling at the DSAC (64.62 ± 18.30 days postpartum) than at Urbana (57.42 ± 15.20 days postpartum). In opposition to these findings, Troxel et al. (1983) in a previous study conducted at DSAC observed a lower degree of cyclicity (51%) for suckled beef cows at similar intervals following parturition and the average days postpartum reported for anestrous cows (57.3 ± 1.4) did not differ ($p>.05$) from cyclic cows (65.5 ± 1.6). Several environmental factors may be accounted for this disagreement. Inherent improvements on the management of the farm over the years might have played a role in reducing stress around parturition and thus minimizing variations in body condition and body weight with hastening effects on the resumption of postpartum ovarian activity (Dunn and Kaltenbach, 1980). Unfortunately, body condition scores of the experimental cows are not available and proper comparisons

can not be made with the results of Troxel et al. (1983), who reported an average body condition of 4.5 ± 0.1 before timed breeding.

The present study was not concerned with the reduction of postpartum intervals. It was assumed that most experimental cows would have already resumed ovarian activity by the time of implant insertion. The cows were 62.78 ± 17.51 days since their previous calving. Therefore, the involution process of uterus should have already completely taken place (López-Gatius and Camón-Urgel, 1989; Archbald et al., 1990; Glanvill and Dobson, 1991; Morton et al., 1992). In addition, it has been reported that the average time from parturition until first ovulation ranges from 36 to 71 days in beef cows (Wettemann, 1980; Mee et al., 1991). In this study, it seemed to be reasonable to anticipate a greater number of cycling than anestrous cows because by the time of implants insertion cows were closer (62.78 ± 17.51 days) to the upper than the lower limit of the previously reported range for resumption of ovarian activity. Nonetheless, it is acceptable that a small number of cows were still in anestrous because postpartum intervals greatly vary and reach, even under satisfactory management, more than 100 days (Dunn and Kaltenbach, 1980; Wettemann, 1980; Perry et al., 1991). In this study, there was no separation of calves and dams. Therefore, blockade effect from suckling towards the hypothalamic-hypophysial axis was to be expected (Wagner and Oxenreider, 1971; Wettemann, 1980; Troxel et al., 1983; Valle, 1986; Roche and Boland, 1991), especially in late-calved cows, which had the

shorter intervals since parturition and were suckled more frequently by their still monogastric and dependent offspring.

The efficiency of the luteolytic action of the $PGF_{2\alpha}$ is accurately determined based on changes in the peripheral concentration of progesterone (Kesler and Favero, 1990). Another way to assess luteolytic effects of $PGF_{2\alpha}$ is by observing estrus behavior within a determined period of time after treatment. However, $PGF_{2\alpha}$ and its analogues only consistently induce luteolysis during the diestrus, i.e., between days 6 and 17 of the estrous cycle (Smith et al., 1985; Whittier et al., 1986; Thibodeaux et al., 1992). Thus, the assessment of progesterone profiles in the plasma or in the serum represents a more accurate method to determine the presence of a functional corpus luteum (Mutiga et al., 1993) and ultimately the occurrence of luteolysis (Kesler and Favero, 1990). Peripheral levels of progesterone sharply decline within 24 hours (Hafs et al., 1974), achieve basal levels of less than 1 ng/ml within 36 to 48 hours (Garcia-Winder and Gallegos-Sanchez, 1991; Logue et al., 1991; Thibodeaux et al., 1992; Mutiga et al., 1993) and assume concentrations between 0.60 and 0.70 ng/ml at the fifth day after $PGF_{2\alpha}$ administration (Thibodeaux et al., 1992).

The efficiency of the luteolytic action of the $PGF_{2\alpha}$ is summarized in the Table 2-3. Induced regression of corpus luteum occurred in 38 cows out of 58 (65.5%) with functional corpus lutea at $PGF_{2\alpha}$ administration. On the other hand, spontaneous regression was observed in 24 out of 59 (40.7%) control cows with corpus

luteum. Therefore, the provoked induction of luteolysis led a greater number ($p<.05$) of cows to undergo a drop in their serum level of progesterone than did the spontaneous luteolysis during the same period of time.

Washburn and Dailey (1987) pointed out that the failure of corpus luteum to regress after $PGF_{2\alpha}$ treatment limits the synchronization of estrus. Logue et al. (1991) reported a frequency of 46.8% (18/37) heifers with functional corpus luteum at the time of a 7.5 mg injection of luprostiol ($PGF_{2\alpha}$-analogue) and the luteolytic response was 77.8% (11/18). On their turn, Glanvill and Dobson (1991) verified that 56 out of 90 (62%) cows had active corpus luteum at the time of a 25 mg $PGF_{2\alpha}$, as dinoprost tromethamine, injection and 91% (51/56) underwent luteolysis. The results herein reported (65.5% of luteolysis) are below the range previously mentioned but still consistent and significantly ($p<.05$) higher than spontaneous regression (40.7%) noted for control cows. As a matter of fact, the efficiency and rate of luteolysis are affected by the stage of estrous cycle when administration is proceeded (Tanabe and Hann, 1984) and high peripheral levels of progesterone cannot be always attributed unequivocally to the presence of an actively secretory luteal tissue. In actuality, Logue et al. (1991) using an ultrasound scanning device demonstrated that 11% (2/18) of regressing corpora lutea were coupled with high plasma concentration of progesterone. Therefore, some proportion of $PGF_{2\alpha}$-treated cows taken as susceptible to luteolysis (n=58),i.e. progesterone concentration $\geq$1.5 ng/ml at the

time of injection, might be already undergoing demise of corpus luteum and the sampling proceeded five days later would coincide with the luteal phase of the following cycle. Additionally, the dynamics of formation and turnover of $PGF_{2\alpha}$ receptors in the luteal cells throughout the estrous cycle period is still poorly understood (Logue et al., 1991) and individual variations in response to luteolytic agents have been reported (Garcia-Winder and Gallegos-Sanchez, 1991). On the other hand, corpora lutea formed after the first ovulation postpartum and anticipated to be short-lived are not more responsive to $PGF_{2\alpha}$ than the corpora lutea anticipated to have normal life span (Copelin et al., 1988).

The decay in progesterone concentration from levels $\geq$ 1.5 ng/ml to less than 1.0 ng/ml within a five days period in control cows had been expected to be 33.0% and served to define the spontaneous regression of the corpus luteum (Table 2-3). In reality, it has been assumed that a population of cyclic cows is equally and randomly distributed within the period of duration of the estrous cycle. Therefore, 57.1% (12/21) of cows would be on the luteal phase (concentration of progesterone $\geq$ 1.5 ng/ml) five days prior to the synchronizing treatment and 33.3% (4/12) would specifically be at days 13 through 16 of cycle. Cows placed on other days could not show the decay in the progesterone profile within a five day period. However, spontaneous regression took place in 40.7% (Table 2-3) bearing corpus luteum five days before treatment. This surprisingly high rate of luteolysis might have happened due to subnormal luteal function associated with short

luteal phase (Kesler et al., 1980; Troxel et al., 1983; Garverick and Smith, 1986). The proportion of short-lived corpora lutea in postpartum cows has been shown to be inversely related with interval since parturition. Nevertheless, the frequency of appearance of first short cycles is yet high even 60 days postpartum (35%) as emphasized by Blasco and Revilla (1992).

In summary, the randomized selection of 264 suckled beef cows in an average of 62.78 ± 17.15 days postpartum with unknown reproductive status or estrous cycle stage yielded 186 cyclic cows (70.49%) and provided 117 (44.3%) animals at the target stage, i.e. luteal phase, of estrous cycle for $PGF_{2\alpha}$ effects, which manifested in 65.5% of treated (38/58) cows. Thus, resulting in 29.0% (0.443 x 0.665) as the overall efficiency of luteolysis.

Calving rates to the single-timed artificial insemination according to treatments and locations are shown in the Table 2.4.

Overall calving rate was low (28.4%) and differed ($p<.05$) between treatments. The treatment by location interaction was not significant ($p=.410$).

Conception rates to the first service postpartum have repeatedly been reported to be low for estrus synchronized cows (Hixon et al., 1981; Troxel et al., 1983; Mikeska and Williams, 1985; Glanvill and Dobson, 1991; Pleasants and Barton, 1992; Tibary et al., 1992) mainly due to anestrous rate, mistimed insemination (Tanabe and Hann, 1984; Tibary et al., 1992) and abnormal luteal phase (Hixon et al., 1981; Troxel et al., 1983). The same phenomenon happens with non-synchronized cows (Saidudin, 1968 cited

by Casida, 1971; Kiracofe, 1980; Glanvill and Dobson, 1991; Archbald et al., 1992) and this depression in the fertility is assumed to be caused either by the formation of an abnormal corpus luteum or/and failure to maintain luteal tissue (Kiracofe, 1980).

At this point, anestrous cows merit to be considered separately. $PGF_{2\alpha}$ is not capable to induce ovulation in acyclic animals (Brown et al., 1988) and does not play any known role in the formation of corpora lutea of normal life span (Johnson et al., 1992). In addition, $PGF_{2\alpha}$ administrated to both early (<60 days) and late (≥ 60 days) postpartum cows did not hasten the resumption of ovarian activity (López-Gatius and Camón-Urgel, 1989; Archbald et al, 1990; Morton et al., 1992). As a result, control and $PGF_{2\alpha}$-treated anestrous cows were expected to behave similarly, and they did. Calving rates were 26.3% (10/38) to control and 22.5% (9/40) to $PGF_{2\alpha}$-treated anestrous cows ($p>.05$).

Occurrence of short luteal phase after first ovulation postpartum can be minimized by progestogen treatment (Kesler et al., 1980; Valle, 1986; Richards et al., 1988; McCartney et al., 1990; Favero, 1992; Troxel et al., 1993) and subfertility associated with estrous cycles lengths of less then 17 days (Hinshelwood et al., 1982) circumvented. Johnson et al. (1992) postulated that the first postpartum exposure to endogenous progesterone perhaps downregulates its own receptors. In this scenario, exogenous progestogens would somehow interfere with this mechanism. Actually, norgestomet shows greater affinity for uterine specific progesterone binding sites than progesterone

itself (Moffatt et al., 1993). Another possibility raised by Garcia-Winder et al. (1987) is that norgestomet enhances the life span of the induced corpus luteum by increasing the frequency of LH pulses and thereby influencing maturation of ovarian follicles and favoring the normal ontogeny of luteal cells for the synthesis and secretion of progesterone (Mee et al., 1991). However, this hypothesis seems to be refutable by recent findings published by Burns et al. (1993). The assumption that norgestomet implants indeed minimized the occurrence of short-lived corpora lutea after ovulation evokes the search for other explanatory reasons for the low calving rates observed in anestrous cows (24.3% = 19/78). Several causes have been pointed out, such as: lack of ovulation (Beal et al., 1984; King et al., 1988), irregular or abnormal follicular growth before the first ovulation (Mikeska and Williams, 1988; Perry et al., 1991) unbalanced LH secretion following norgestomet implant withdrawal (Hixon et al., 1981), delayed corpora lutea function (King et al., 1986; Favero et al., 1988), reduced concentration of progesterone during luteal phase subsequent to synchronized estrus which alters the uterine environment and reduces embryonic life span (King et al., 1986), or/and asynchronism between onset of estrus, LH pulse and ovulation leading to mismatch between ovulation and insemination (Mikeska and Williams, 1988).

Troxel et al. (1983) had reported a first-service pregnancy rate of 11% for anestrous suckled postpartum beef cows after synchronization with prostaglandins. However, the pregnancy rate

increased to 27% when cows with abnormal luteal phases were removed.

Brink and Kiracofe (1988) found nearly 30% conception rate in anestrous postpartum cows synchronized with Syncro-Mate B and fertility was not affected by presynchronization status. These findings give support to the effects of norgestomet on the prevention of the formation of abnormal luteal tissue (Kesler et al., 1980; Troxel and Kesler, 1984; Copelin et al., 1988; Richards et al, 1988; Mee et al., 1991; Coy and Garcia-Winder, 1991).

In spite of the well-documented properties of progestogen-estrogens combinations to promote ovulations in anestrous cows (Odde, 1990) fertility levels are frequently reported to be higher for cyclic than anestrous cows (Miksch et al., 1978; Troxel et al., 1983; Beal et al., 1984; Ghallab et al., 1984; Whittier et al., 1986). Nonetheless, calving rates observed in the present trial only tended ($p>.50$) to be higher for cyclic cows (30.1%) than for anestrous cows (24.3%) and no significant interactions were detected with location ($p=.847$) or treatment ($p=.662$). Similar findings had been reported by Hixon et al. (1981).

Results reported by Brink and Kiracofe (1988) are consistent and close to the overall calving rate obtained in this trial (28.4%), and thereby it seems appropriate to infer that both systems of norgestomet delivery, hydron and silicone based norgestomet implants, behave similarly and provide equivalent biological responses in suckled postpartum anestrous beef cows.

Progesterone secreted at the time of implantation was used as indicative of the presence of functional corpus luteum and no significant differences ($p>.05$) were found in calving rates for cows bearing luteal tissue (30.4%) compared to those with absence of corpus luteum (27.2%). However, it deserves mention the fact that low serum concentration of progesterone ($\leq$1.5 ng/ml) was expected to occur in postpartum anestrous cows and cyclic cows at estrus, metestrus and proestrus, as well.

The effects of the demise of the corpus luteum is presented in the table 2-5. Overall, the demise of corpus luteum led to lower calving rate ($p<.05$), which might indicate that cows in estrus or metestrus had depressed fertility to the appointed artificial insemination after estrus synchronization. However, the $PGF_{2\alpha}$-induced corpus luteum regression severely repressed fertility (7.9%) whereas the spontaneous regression of corpus luteum did not show the same effect and in fact, tended ($p=.47$) to enhance fertility (50.0%) compared to cows which corpora lutea persisted (45.7%). The presence of corpus luteum in cyclic cows (n=102) did not significantly influence calving rate (30.4%) when compared to the 29.8% of calving rate noted to cyclic cows with no corpus luteum (n=84). These findings completely agree with a previous experiment conducted by Richards et al. (1990), who verified that presence of corpus luteum at the time of synchronizing treatment alters the time to onset of estrus but does not affect significantly pregnancy rates.

Based on the facts that low peripheral levels of progesterone, spontaneous regression of corpus luteum prior to the synchronized estrus (Table 2-5) and absence of corpus luteum at the time of implantation did not reduce ($p>.05$) calving rate of postpartum cyclic cows, it is reasonable to infer that the depressed fertility observed after the estrus synchronization protocol used in this trial was caused by the forced regression of the corpus luteum leading cows to metestrus at Syncro-Mate B treatment. This conclusion disagrees in some extent with the findings reported by Hafs et al. (1974) that found no residual influences of prostaglandin administration on hormone profiles of subsequent estrous cycles. In addition, Gyawu et al. (1991) reported that ovulation cycles were normal in length and progesterone peak concentration at luteal phase allowing the conclusion that corpus luteum function was normal after estrus synchronization with a Syncro-Mate B-$PGF_{2\alpha}$ combination.

The choice of implanting animals on the 5th day after $PGF_{2\alpha}$ injection was made in an attempt to minimize the effects of asynchronism on cyclic cows and also to bring them to early estrous cycle (<11 day) with the expectation that luteolytic action of estradiol valerate and thereby control of luteal function is more efficient during the first half of the estrous cycle.

Table 2-6 presents the effect of the stage of the estrous cycle at initiation of Syncro-Mate B treatment on the fertility of cyclic cows. The outcome of this experiment shows significant influence of the stage of estrous cycle on calving rate of

postpartum suckled beef cows. Higher fertility ($p<.05$) for cows treated on the second (35/79 = 44.3%) than cows on the first (22/107 = 20.6%) half of the estrous cycle was observed. In opposition, Mikeska and Williams (1988) did not find differences in conception rates between heifers synchronized with Syncro-Mate B before day 11 and those started later in the cycle. Similarly, pregnancy rates after estrus synchronization with a combination estradiol benzoate-progesterone were not different between cows treated on days 0 to 10 (27.2%) and on days 11 to 22 (30.7%) of the estrous cycle (Gyawu et al., 1991).

Several reports have shown that stage of estrous cycle at the time of insertion of the implant affect conception rate. However, experimental outcomes are conflicting (Brink and Kiracofe, 1988; Pratt et al., 1991; Fanning et al., 1992).

Inumerous studies agrees with these results for several reasons. The presence of a functional corpus luteum at implant removal impedes pregnancy (Hixon et al., 1981; Ghallab et al., 1984; Whittier et al., 1986; Gyawu et al., 1991; Logue et al., 1991) and this fact is associated either with the failure of the estradiol valerate to induce incomplete demise of a pre-existing corpus luteum or with the occurrence of ovulation while implant is kept in situ (Hixon et al., 1981; Pratt et al., 1991; Fanning et al., 1992; Twagiramungu et al., 1992; Burns et al., 1993).

Brink and Kiracofe (1988) found higher fertility in cows treated early in the estrous cycle (<11 days) and concluded by the better luteolytic efficiency of estradiol valerate during that

period. Other reports affirm that Syncro-Mate B treatment is less effective in synchronizing estrus when administered at metestrus (Pratt et al., 1991; Fanning et al., 1992; Burns et al., 1993). The apparent inability to prevent corpus luteum development (Pratt et al., 1991; Fanning et al., 1992) and induce complete luteolysis (Burns et al., 1993) over the period that implants are in situ generates elevated levels of circulating progesterone at the time of implant removal with consequent low estrus response (Pratt et al., 1991; Fanning et al., 1992), asynchrony (Mikeska and Williams, 1988) and reduced fertility to the timed insemination. Attempts have been made to enhance the control of luteal phase with Syncro-Mate B. Increased doses of estrogens at the time of treatment did not reduce the frequency of cows with functional corpus luteum at implant removal and estrus response did not differ between cows receiving between 5 and 9 mg of estradiol valerate (Pratt et al., 1991). On the other hand, Fanning et al. (1992) verified that control of luteal function using Syncro-Mate B is enhanced by higher dosages of injectable norgestomet (6mg) and as a result fewer cows with functional corpus luteum were observed at implant removal, more cows exhibited estrus within five days of the end of treatment and a higher 5 days pregnancy rate (66%) was obtained when compared to doses of 3 mg (38%) and 4.5 mg (45%). These results are supported by the fact that Syncro-Mate B administered on day 2 of the cycle led to inhibition of LH secretion and regression of corpus luteum (Burns et al., 1993). Nonetheless, the LH decrease did not totally abolish luteal tissue development

because some cows (58.3%) showed functional corpus luteum subsequently to treatment.

The secretion of progesterone in cows treated at early stages of estrous cycle and that do not have functional corpus luteum at implant removal is characterized by a pattern of marked suppression noticeable 4 days after implant insertion (Kesler et al., 1984; Burns et al., 1993). On the other hand, peripheral levels of progesterone are maintained at high levels for longer in cows treated at mid-cycle (day 10) or on day 3 of cycle. In these circumstances corpus luteum is present at implant withdrawal (Kesler et al., 1984; Burns et al., 1993).

The low calving rates observed in this trial for both control and $PGF_{2\alpha}$-treated cows (Table 2-4) are within ranges previously reported (Odde, 1990) and can be partially attributed to the stage of estrous cycle when synchronizing treatment was imposed (Table 2-6). In agreement with other reports, cows treated towards the end of the estrous cycle may have the benefit of undergoing natural luteolysis and therefore show an enhanced estrus response within the few days after implant removal (Pratt et al., 1991; Fanning et al., 1992). Furthermore, most of reports have computed fertility to the first five days after the treatment and additional inseminations were allowed to occur after the timed artificial insemination and based on clinical signs of estrus (Pratt et al., 1991; Fanning et al., 1992). Higher fertility rates reported do not invalidate the findings herein described, once fertility in

this experiment was calculated exclusively based on calvings from a single timed artificial insemination.

The importance of the stage of estrous cycle at initiation of synchronizing treatment is emphasized by results of this experiment. In addition, individual responses to different treatments (Garcia-Winder and Gallegos-Sanchez, 1991; Pratt et al., 1991; Fanning et al., 1992) imposed at same stages of the cycle suggests that even better control of luteal function is required to adequately and effectively synchronize and induce estrus with normal fertility in postpartum suckled beef cows.

The pretreatment with $PGF_{2\alpha}$ depressed the fertility to the timed artificial insemination (Table 2-5). Prostaglandin treatments have the ability to synchronize luteolysis but not necessarily to synchronize proestrus, due mainly to the increased rate of ovarian follicle development and maturation after induced luteolysis (Kerr et al., 1991). Follicular dynamic is also affected by estradiol valerate, which suppresses the growth of dominant follicle and leads to early emergence of the next follicular wave in heifers treated on day 1 of the estrous cycle (Bo et al., 1993). Treatment on day 3 resulted in delayed emergence of the next wave and treatments initiated after the dominant follicle has completed its growth (day 6) did not alter the static or regressing phases (Bo et al., 1993).

As observed, the effects of commencing treatments on day 1 or day 3 are somewhat opposite in regard to follicular growth and the prostaglandin administration five days before the synchronizing

procedure does not ensure a tight concentration of cows at any particular stage of the induced proestrus, estrus or metestrus at the time of Syncro-Mate B treatment. Therefore, a proportion of cows at luteal phase that receive $PGF_{2\alpha}$ (n=58) and rapidly underwent luteolysis (n=38) could possibly be between days 1 and 3 of the cycle and follicular dynamics would thereby be compromised. This is supported by the fact that onset of estrus ranges from 44 to 72 hour (Hafs et al., 1974; Tanabe and Hann, 1984) after $PGF_{2\alpha}$ injection.

CONCLUSIONS

Calving rate of postpartum suckled beef cows submitted to estrus synchronization with a 6 mg norgestomet silicone implant kept in situ for 9 days with a 3 mg of norgestomet and 5 mg of estradiol valerate injection at the time of implant insertion was lower ($p<.05$) for cows treated with 25 mg of $PGF_{2\alpha}$, as dinoprost tromethamine, five days before. Calving rate was significantly ($p<.05$) affected by days postpartum and stage of estrous cycle at implant insertion. Pre-synchronization ovarian status, location, presence of corpus luteum at implant insertion and interactions among them did not influence ($p>.05$) fertility to the single-timed artificial insemination. In summary, calving rate was depressed when Syncro-Mate B treatment commenced during the first half of estrous cycle and cows treated between days 1 and 5 of the cycle showed the poorest fertility.

TABLE 2-1. NUMBER OF CYCLIC COWS AT THE BEGINNING OF THE ESTRUS SYNCHRONIZATION TREATMENT ACCORDING TO LOCATION AND PRE-TREATMENT.

	Cycling[1]					
	DSAC[2]		Urbana		Overall	
	n	%	n	%	n	%
Control	74	74.0	21	63.6	95	71.4
$PGF_{2\alpha}$	73	74.5	18	54.5	91	69.5
Total	147	74.2[a]	39	59.1[b]	186	70.4

[1] Cows were considered cycling when serum progesterone concentration was $\geq$ 1.5 ng/ml at least once in a series of three collections comprised of a 16 day interval.

[2] Dixon Springs Agricultural Center, Simpson, IL.

[a,b] Figures in the row with different superscript differ ($p<.05$).

TABLE 2-2. INTERVAL SINCE PARTURITION TO INITIATION OF THE ESTRUS SYNCHRONIZATION PROGRAM[1].

Effects	N[4]	Interval (days) (X±s)
Location		
DSAC[2]	193	64.62 ± 18.30[a]
Urbana	66	57.42 ± 15.20[b]
Treatment[3]		
Control	131	63.92 ± 17.55[a]
$PGF_{2\alpha}$	128	61.62 ± 18.06[a]
Ovarian activity		
Cyclic	181	67.65 ± 16.12[a]
Anestrous	78	51.50 ± 16.44[b]

[1] Means with different superscript letters differ ($p<.01$).

[2] Dixon Springs Agricultural Center, Simpson, IL.

[3] Treated cows received a $PGF_{2\alpha}$ injection five days before the insertion of implants.

[4] Data were not available for five cows.

TABLE 2-3. EFFICIENCY OF THE INDUCTION OF LUTEOLYSIS BY MEANS OF 25 mg OF $PGF_{2\alpha}$ INTRAMUSCULARLY ADMINISTERED IN POSTPARTUM BEEF COWS.

Treatment	N	Active CL[1]		CL regression[2]	
		n	%	n	%
Control[3]					
DSAC	100	49	49.0	22[b]	46.9
Urbana	33	10	30.3	02[b]	20.0
Total	133	59	44.4	24[b]	40.7
$PGF_{2\alpha}$					
DSAC	98	49	50.0	33[a]	67.3
Urbana	33	09	27.3	05[ab]	55.5
Total	131	58	44.3	38[a]	65.5
Overall	264	117	44.3	63	53.8

[1] Corpus luteum was considered active when serum progesterone concentration was ≥ 1.5 ng/ml at the time of $PGF_{2\alpha}$ administration.

[2] Corpus luteum regressed when concentration of progesterone in the serum dropped to less than 1.0 ng/ml, five days after the $PGF_{2\alpha}$ administration.

[3] Control cows did not receive any injection and corpus luteum regression was considered as a spontaneous event.

[a,b] Figures within the same column with different superscripts differ ($p<.05$)

TABLE 2-4. EFFECT OF THE ADMINISTRATION OF $PGF_{2\alpha}$ PRIOR TO THE ESTRUS SYNCHRONIZATION PROCEDURE ON THE CALVING RATE OF POSTPARTUM BEEF COWS ACCORDING TO LOCATION.

	Treatment								
	Control			$PGF_{2\alpha}$			Overall		
	N	Calving		N	Calving		N	Calving	
		n	%		n	%		n	%
Location									
DSAC	100	32	32.0	98	25	25.5	198	37	28.8
Urbana	33	12	36.4	33	06	18.2	66	18	27.3
Total	133	44	33.1[a]	131	31	23.7[b]	264	75	28.4
Cyclicity									
Cyclic	95	34	35.8	91	22	23.1	186	56	30.1
Anestrous	38	10	26.3	40	09	22.5	78	19	24.3

[1] There was no statistically significant differences ($p>.05$) between locations or between cyclicity status.

[a,b] Means with different superscript letters differ ($p<.05$).

TABLE 2-5. EFFECT OF CORPUS LUTEUM DEMISE[1] BEFORE THE INITIATION OF THE ESTRUS SYNCHRONIZATION PROGRAM[2] ON THE CALVING RATE OF POSTPARTUM SUCKLED BEEF COWS.

	Corpora lutea status						
	Active[3]	Demised			Persisted		
			calving			calving	
		n	n_d	rate (%)	n	n_p	rate (%)
Control	59	24	12	50.0[a]	35	16	45.7[a]
$PGF_{2\alpha}$	58	38	03	7.9[b]	20	08	40.0[a]
Overall	117	62	15	24.2[b]	55	24	43.6[a]

[1] Corpus luteum regressed when concentration of progesterone in the serum dropped from 1.5 ng/ml to less than 1.0 ng/ml since five days before the initiation of the estrus synchronization treatment.

[2] The program consisted of a silicone ear implant containing 6 mg of norgestomet and the injection of 3 mg of norgestomet and 5 mg of estradiol valerate at the time of implant. insertion.

[3] Corpus luteum was considered active when serum concentration of progesterone was equal to or higher than 1.5 ng/ml.

[4] Means with different superscript letters differ ($p<.05$).

TABLE 2-6. CALVING RATES OF POSTPARTUM BEEF COWS SYNCHRONIZED WITH SYNCRO-MATE B® ASSOCIATED WITH OR WITHOUT $PGF_{2\alpha}$ PRETREATMENT ACCORDING TO CYCLICITY[1] AND STAGE OF THE ESTROUS CYCLE[2].

Cyclicity	Control		$PGF_{2\alpha}$		Combined	
	Calving		Calving		Calving	
	n	(%)	n	(%)	n	(%)
Anestrous	10/38	26.3	09/40	22.5	19/78	24.3[b]
Stage of the cycle[3]						
day 1 to 5	01/04	25.0	07/45	15.6	08/49	16.3[b]
day 6 to 10	07/32	21.2	07/26	26.9	14/58	24.1[b]
day 11 to 17	15/35	42.8	08/20	40.0	23/55	41.8[a]
day 18 to 0	12/24	50.0	-	-	12/24	50.0[a]

[1] Cows were considered cycling when serum progesterone concentration was ≥ 1.5 ng/ml at least once in a series of three collections comprised of a 16 day interval.

[2] Estrous cycle length was considered to be 21 days with luteal phase (progesterone concentration ≥ 1.5 ng/ml) between days 6 and 17 of the cycle.

[3] Stage of the cycle at Syncro-Mate B® treatment.

[a,b] Means with different superscript letters differ ($p<.05$)

CHAPTER III.

SECRETION OF NORGESTOMET FROM SILICONE (POLYDIMETHYLSILOXANE) IMPLANTS AND THE SUPPRESSION OF ESTRUS IN BEEF COWS

SUMMARY

The daily secretion rate of norgestomet from polydimethylsiloxane (silicone) implants impregnated with either 6 mg or 8 mg of norgestomet was determined by validated *in vitro* and *in vivo* assays. In addition, the ability of these norgestomet impregnated silicone implants to suppress estrus in postpartum beef cows was assessed. The combined findings made it possible to establish a minimal daily implant secretion of norgestomet that would effectively suppress estrus behavior in beef cows. The *in vitro* assay consisted of a bovine serum culture system maintained at 37 °C for 16 days. Culture medium was changed every 24 hours. Serum was individually extracted for norgestomet and norgestomet concentration determined spectrophotometrically. Norgestomet secretion rate was determined on a daily basis and used to generate regression equations. The *in vivo* secretion was determined through complete extraction of norgestomet from new implants (total content) and implants left *in situ* for 16 days (*in vivo* secretion). The difference was considered the amount secreted *in vivo*. In addition, determination of remaining norgestomet in the implants previously used in the *in vitro* assay was performed (*in vitro* secretion). The behavioral trial involved 59 beef cows which were initially synchronized with the Syncro-Mate B program and

artificially inseminated 48 after implant removal. On day 5 the cows were randomly allotted in two groups. Twenty nine cows received a 6 mg norgestomet implant and 30 cows received a 8 mg implant. Estrus behavior was determined twice daily until the withdrawal of the 2nd implant. Of 16 non-pregnant cows implanted with 6 mg norgestomet implants, three (18.8%) displayed breakthrough estrus. None of the cows implanted with 8 mg norgestomet implants expressed estrus behavior over the 16 day period. Based on these results, it is inferred that secretion of 136 μg norgestomet daily effectively suppressed estrus in beef cattle.

INTRODUCTION

In cyclic cows, the time of estrus is controlled by the secretion of progesterone from the corpus luteum. Progesterone exerts negative feedback on LH release from the pituitary gland in such a way that maturation of preovulatory follicles and subsequent ovulation are inhibited until progesterone declines at the time of corpus luteum regression. Therefore, controlling the life span of the corpus luteum makes it possible to predict the time of estrus and ovulation (Hansel and Convey, 1983). This is the theoretical basis behind the procedure known as synchronization of estrus and ovulation.

Progestogens are natural or synthetic steroids with molecular resemblance with progesterone (Gore-Langton and Armstrong, 1988). Norgestomet (17α-acetoxy-11 ßmethyl-19-nor-preg 4-ene 3,20 dione)

is a potent progestogen and its continuous administration can mimic some of the biological effects of progesterone. The administration of norgestomet in cattle, alone or in combination with other drugs has been capable to suppress estrus behavior (Wishart, 1972), induce ovulation (Brink and Kiracofe, 1988), synchronize estrus (Wiltbank and Gonzalez Padilla, 1975), synchronize calvings (Janszen et al., 1990), improve weight gain of beef heifers (Hill et al., 1992) and maintain pregnancy (Favero et al., 1990). However, the effectiveness of norgestomet treatment relies upon a required minimal circulating level. Moreover, the relatively rapid metabolic turnover of steroids in the body of the animals (Gore-Langton and Armstrong, 1988) makes daily injections schedules impractical. Fortunately, steroids are able to pass through polymers into in vitro media (Dziuk and Cook, 1966; Karsch et al., 1973; Christensen and Kesler, 1984; Kesler and Favero, 1989; Favero et al., 1990) and in vivo biological systems (Dziuk and Cook, 1966; Kesler and Favero, 1989; Favero et al., 1990). This property made possible the development of sustained-release steroid implants, which in turn facilitates their evaluation and use under field conditions on a large scale.

It should be mentioned that among available polymers, polydimethylsiloxane (silicone) is considered to be non-antigenic and non-irritating (Dziuk and Cook, 1966) and its high permeability to steroids and excellent diffusion properties gives silicone elastomers a high degree of biocompatibility (Sun et al., 1986). Therefore, the development of drug-filled silicone capsules made

feasible the chronic administration of steroids. In spite of the polydimethylsiloxane characteristics, a norgestomet implant manufactured with a hydron polymer, polyethyleneglycol methacrylate, is commercially available. Nonetheless, the kinetics of drug permeation differs according to the elastomer employed (Ghannam et al., 1986) because solubility and partition coefficient of drugs are affected by the structural characteristics of the polymer (Ghannam et al., 1986; Sun et al., 1986). Therefore, the same steroid might behave differently when dispersed into distinct elastomer types. As a matter of fact, Kesler and Favero (1989) and Favero (1992) verified that the use of polydimethylsiloxane norgestomet implants in substitution to the commercially found polyethylene glycomethacrylate improved the outcome in fertility of the Syncro-Mate B program.

Silicone norgestomet implants proved to be not only efficient in improving fertility levels of the Syncro-Mate B program, but also have been tested for resynchronization schedules, where a second implant is placed <u>in situ</u> some days after the initial artificial insemination. Such a practice allows a second opportunity for timed artificial insemination and as result, the overall fertility achieved through the estrus synchronization program is increased and a more tightly synchronized calving season is to be expected (Favero, 1992). However, resynchronization protocols have some constraints. It is well known the detrimental effects of long exposure to progestogens on the fertility of postpartum cows (Odde, 1990). Thus, the interval from the initial

artificial insemination and the withdrawal of the second silicone norgestomet implant shall not exceed the natural length of the luteal phase, i.e., 21 days on average.

As mentioned earlier, an adequate circulatory supply of norgestomet is demanded to effectively suppress estrus behavior. This supply is given by the daily release of the steroid from the implant. The release of steroids from silicone implants is governed by Fick's law of diffusion with the flux of diffusion being affected by a diffusion coefficient, membrane surface area, thickness of the membrane and concentration gradient across the diffusion path in the membranes (Kesler, 1989).

The main goals of this study are to determine the secretion rate of norgestomet daily released from polydimethylsiloxane implants impregnated with different amounts of the steroid and compare the secretion rate with the behavioral profile of cows submitted to a resynchronization regimen using the same types of implants. Furthermore, a threshold for the norgestomet effective suppressing activity towards estrous behavior is to be established.

MATERIAL AND METHODS

IN VITRO ASSAY FOR NORGESTOMET RELEASE. The release rate and the mass of norgestomet daily delivered from two different types of cylindrical polydimethylsiloxane implants were assessed through a validated *in vitro* system (Kesler and Favero, 1989; Favero et al., 1990). The main physical characteristics of the implants are shown in the table 3-1.

Four identical implants from each type (Table 3-1) were individually placed in 16 x 100 mm glass culture tubes. The incubation medium was comprised of 10.0 ml of pooled bovine serum enriched with 10 μl of a 10% solution of thimerosal. The incubation was performed in water bath at 37 °C and the incubating periods lasted 24 hours after which implants were removed from culture medium and placed in a new culture tube with the same characteristics above described. This procedure took place until 16 days of incubation have been completed. Norgestomet was extracted from each serum sample according to technique described by Kesler et al. (1990) for progesterone and having slight modifications. A serum sample aliquot of 200 μl was added to a 16 x 150 mm glass culture tube. Following the addition of 2.0 ml of petroleum ether, the sample was vigorously shaken on a mechanical shaker for 30 seconds. The mixture was stored at -10 to -20°C and the organic phase was decanted into a 12 x 75 mm glass culture tube. The sample was then placed into a 40 to 52°C water bath. Once dry the norgestomet was reconstituted with 1.0 ml of absolute (100%) ethyl alcohol and vortexed for three seconds before the absorbance reading. Amount of norgestomet present in the sample was determined by ultraviolet absorption on a spectrophotometer at a wavelength of 240 nm. The obtained value was then compared to standard values, which had generated the following linear equation:

$$Y = 22.0445\ X - 0.1302$$

where Y = mass of norgestomet (μg)

and X = absorbance at 240 nm wavelength.

A correction factor was determined to take into account the volume of the system (10.0 ml), the dilution employed (1:5) and the efficiency of extraction or recovery (87 % recovery; 1.1427). The background concentration of norgestomet was also determined (1.4 μg/10.0 ml) and discounted. As a result, the complete equation for the determination of daily release of norgestomet became:

$$Y = [(22.0445\ X - 0.1302) \times 57.135] - 1.4$$

Where: Y = μg of norgestomet released

X = absorbance at 240 nm

Additionally, daily rate of release was determined and it has been defined as:

$$\text{Release rate } n^{th} \text{ day} = \frac{\text{Mass of norgestomet released on day } n^{th}}{\text{Mass of norgestomet left on day } (n-1)^{th}}$$

IN VIVO ASSAY FOR NORGESTOMET RELEASE. The *in vivo* secretion of norgestomet from two different types (6 mg loaded and 8 mg loaded) of polydimethylsiloxane implants were assessed through a validated *in vivo* system (Kesler and Favero, 1989; Favero et al., 1990).

Four cows were implanted with two implants each in the ears (one 6 mg and other 8 mg norgestomet). The implants were left *in situ* for 16 days. Upon removal total content of norgestomet remaining was determined. In addition, norgestomet content of four implants of each type that had previously been submitted to the *in vitro* assay and three non-used implants of each type was

determined. Implants were individually placed in 16 x 100 mm glass culture tubes. Each tube received 10.0 ml of methanol and was tightly covered with parafilm and incubated in water bath at 37 °C for six days. Amount of norgestomet extracted was determined as previously described for the *in vitro* assay with the volume of the system being corrected individually to each tube. As a result, the equation for the determination of norgestomet recuperated became:

$$Y = [(22.0445\ X - 0.1302) \times 40.0] \times V$$

Where: Y = μg of norgestomet recuperated

X = absorbance at 240 nm

40.0 = dilution employed (25 μl extracted/975 μl methanol)

V = volume measured after 6 days of incubation

BEHAVIORAL TRIAL. Fifty-nine postpartum beef cows were synchronized with the conventional Syncro-Mate B program. The synchronization protocol consisted of an intramuscular injection of 5 mg estradiol valerate and 3 mg norgestomet in sesame oil (and 10% benzyl alcohol) carrier associated to the subcutaneous insertion of a 6 mg norgestomet implant on the convex surface of the ear. Implants were left *in situ* for nine days and timed artificial insemination took place approximately 48 hours after implants withdrawal. On the 5th day since the initial artificial insemination, cows were randomly assigned in two groups and were re-implanted with an additional polydimethylsiloxane implant, which was left *in situ* for 16 days. Twenty nine cows received a 6 mg

norgestomet implant, whereas 30 cows were implanted with a 8 mg norgestomet capsule (Table 3-1).

A cow was considered in estrus when standing to be mounted by other females. An experienced herdsman visually checked cows for estrus twice a day, early morning and early evening, since the day of the insertion of the 2nd implant until the moment of its withdrawal 16 days later.

STATISTICAL ANALYSIS. Norgestomet levels are presented as means (± standard deviation). Statistical tests included analysis of regression for the daily release of norgestomet according to each type of implant studied, analysis of variance for repeated measures to determine time-wise differences in the mass of norgestomet delivered and its rate of release. The Chi-square test was employed to compare observed frequencies of cows in estrus (Steel and Torrie, 1980). All statistical analysis were performed through the SAS statistical program.

TABLE 3-1. POLYDIMETHYLSILOXANE - NORGESTOMET IMPREGNATED IMPLANTS.

Type of implant	Length (mm)	Diameter (mm)	Norgestomet load (mg)[1]	Initial Surface Area (mm^2)
6 mg	18.5	2.67	6.0	166
8 mg	25.0	2.67	8.0	221

[1] Theoretical load.

RESULTS AND DISCUSSION

Norgestomet passed through polydimethylsiloxane membrane into serum medium as anticipated. Similar results had been described earlier not only for norgestomet (Kesler and Favero, 1989; Favero et al., 1990) but also for other natural and synthetic steroids (Dziuk and Cook, 1966; Karsch et al., 1973; Christensen and Kesler, 1984; Kesler, 1989). Daily secretion of norgestomet in vitro is shown in Figure 3-1. Norgestomet released into serum medium significantly differed ($p<.05$) among implants when a temporal (time-wise) comparison was performed. 8 mg norgestomet implant always secreted a higher amount of the hormone than did the 6 mg norgestomet implant. It could not be inferred whether the increase of surface area or the greater amount of steroid in the 8 mg norgestomet implant was the responsible for the higher level of norgestomet delivery observed to that type of implant. Actually, the increase in surface area (55 mm^2) was accompanied by proportional increase in mass of norgestomet impregnated (2000 μg) in such a way that the ratio between initial mass and initial surface remained the same for both types of implants (36.2 $\mu g/mm^2$). This outcome is supported by previous information available from other studies which demonstrated that progesterone and progestogen release rates are increased as the surface area of the implant increases (Dziuk and Cook, 1966; Turner et al., 1981; Kesler, 1989) and apparently are not affected by weight of steroids in the capsule (Dziuk and Cook, 1966). These findings seemed to agree with the results herein reported because the relative weight

(density) of steroids in the implants was designed to be the same (approximately 57 $\mu g/mm^3$). Therefore, as implants had the same "density" and same initial ratio mass/surface, it seems to be likely that the net increase in norgestomet delivery (Table 3-2) was due to the increase on the surface area of the implant. As a matter of fact, Turner et al. (1981) concluded that the amount of steroid daily released from estradiol 17ß silicone implants depended on surface area and that an increase in length of the capsules led to a linear increment of secretion. In addition, Kesler (1989) proved that increasing the length of silicone implants (surface area) impregnated with testosterone and its esterified salts linearly increased plasma testosterone concentrations in beef cows implanted with capsules of different sizes.

Figure 3-2 shows the cumulative mass of norgestomet released (μg) the implants used in this study. The cumulative mass secreted from 6 mg norgestomet implant observed in the present trial (Figure 3-2) fully agrees with previous findings of other workers. Total delivery of 6 mg norgestomet hydron implants kept 9 and 16 days *in situ* was reported to be respectively, 2.36 and 3.18 mg (Spitzer et al., 1978). Kesler and Favero (1989) obtained similar *in vivo* secretions of both 6 mg hydron (2.185 mg) and 6 mg silicone (2.393 mg). No analogous data are available for critical comparisons with the 8 mg of norgestomet loaded implant.

Several reports (Karsch et al., 1973; Spitzer et al., 1978; Turner et al., 1981) used *in vitro* systems of evaluation and

described the release rate as an average between the total amount of steroid secreted and the number of days while implants were kept in place. Spitzer et al. (1978) reported a range of 170 to 200 μg of daily release of norgestomet from 6 mg norgestomet hydron implants. However, the actual delivery rate can not be considered constant over time because as steroid leaves the capsule the surface area, the thickness, the interface and the concentration gradient across donor and acceptor membrane change. In this case, the assumption of constant release overrules Fick's law of diffusion and provides and inaccurate estimation of the secretion.

The pattern of release (% rate) was somewhat constant within type of implant assessed, which means that even with the increment of the membrane layer between donor and acceptor media, the proportionality of deliver was unchanged. Therefore, both types of implants represented fidel systems of sustained release of norgestomet. However, the efficiency of delivery varied between systems. For example, the release rate observed to 8 mg norgestomet implant averaged 6.15 ± 0.46% over the 16 day period and it was smaller than that observed for 6 mg implants (6.70 ± 0.41%). In spite of the difference in the velocity of mobilization (6.15 vs. 6.70%) the 8 mg implant ensured a larger delivery of steroid in a daily basis (Figure 3-1) due to the initial greater amount of steroid sequestered in its core. An analogous reasoning is given to explain the 6 mg implants incapability to continuously release greater amounts of norgestomet over the three last days of incubation. It seemed that the faster release rate noted for the

6 mg implants led to a quick exhaustion of the steroid content in the implant. A similar trend of secretion of norgestomet from hydron implants had been reported by Kesler and Favero (1989), who also observed an initial 3 day period of burst release followed by a 5 day period of steady delivery and ended with an erratic secretion. Nonetheless, it must be emphasized that in the present study the "steady phase" lasted until day 12 and very low in vitro delivery of norgestomet was only observed on days 15 and 16 of incubation (Figure 3-1). As a result, the pattern of secretion seen for the 6 mg cylinder might have been an advantageous feature if that implant had been designed for nine-day long estrus synchronization protocols. On the other hand, the rapid perfusion from the implant opens the light of producing an implant with smaller quantity of norgestomet to be used in 9 day long programs of ovulation control in cattle.

In order to ensure the suppression of estrus behavior, the minimum amount of norgestomet daily released into the circulatory system of a cow is assumed to be 140 μg (CEVA laboratories, Inc., Overland Park, U.S). The use of this value as the real standard threshold for adequate release of norgestomet from the implant would fully ensure the effectiveness of both types of implants over the 16 day period because their lowest weight released only happened on the 16^{th} of incubation and were, respectively 225 μg and 143 μg (Figure 3-1) for 8 mg and 6 mg norgestomet implants.

Analysis of regression was performed and equations to predict the daily release of norgestomet are shown in the Table 3-2, which

also presents the obtained correlation coefficients (r) and coefficients of determination (R^2). The usefulness of the regression equations is related with the fact that it becomes possible to predict the duration of the effectiveness of any of the implants herein assessed. For instance, assuming 140 μg as the threshold for the appearance of the breakthrough estrus, it could be anticipated that the theoretical effectiveness of 8 mg and 6 mg norgestomet implants would not go beyond the 18^{th} and 14^{th} days since insertion respectively.

The outcome of the in vivo assay is summarized in the Table 3-3, which shows the amount of norgestomet present in the implants before (total content) and after a 16 day incubation period (in vitro recuperation) or an in situ 16 day implantation period (in vivo recuperation). As result, in vitro and in vivo secretions were determined by difference, compared between each other and a correction factor for in vitro delivery was determined.

In vitro delivery was more rapid than in vivo secretion. In actuality, the in vitro rate of secretion was 37.8% faster than the in vivo release. It deserves mention the fact that the kinetic behavior observed in the in vivo assay was essentially the same for both types of implants assessed. Furthermore, the results herein obtained confirmed previous findings of Kesler and Favero (1989) who also reported a 30.7% faster in vitro delivery compared to in vivo systems for evaluation of the secretion. Nonetheless, the same authors found different kinetic profile of release for 6 mg implants manufactured with polyethylene glycomethacrilate polymer.

Therefore, the in vitro system initially employed to measure the daily release and the cumulative mass of norgestomet delivered needed to be adjusted for that differentiated kinetic behavior observed in vivo. Figure 3-1 presents the adjusted values. It could be also be concluded that the in vitro assay employed effectively detected relative secretion coming out of the implants because there was no significant difference ($p>.05$) between the 16 day cumulative secretion verified for the in vitro assay and the secretion verified through difference (total content deduced of remaining content) for the in vivo assay (Tables 3-3) thus permitting the reliable adjustment of in vitro values into in vivo corrected secretions (Figure 3-1).

The prediction curves for in vitro release of norgestomet from silicone implants are also depicted in Figure 3-1. The figure not only presents a scale for the observed in vitro secretion but also a scale with the adjusted secretion for the differences found between in vitro and in vivo kinetic behavior.
Figure 3-2 shows the corrected cumulative delivery of norgestomet from both implants.

Cows pregnant to the initial artificial insemination were not considered for behavioral assessment. In addition, implants were lost in one cow from each group and they were not considered to further comparisons. Implant loss rates were 3.4% (1/29) and 3.33% (1/30) to 6 and 8 mg norgestomet implants, respectively. These rates are in agreement with ranges normally reported to polyethylene methylacrylamide amide norgestomet implants (Broadbent

et al., 1992) and to polydimethylsiloxane norgestomet implants (Favero, 1992). Loss of implants may have several mechanisms of occurrence, which were discussed in the chapter II.

Three cows with the 6 mg norgestomet implant exhibited breakthrough estrus. Cows implanted with 8 mg capsules did not display estrus within the 16 day period while implants were in place. Breakthrough estrus has been reported in cows implanted for longer than 9 days with 6 mg norgestomet hydron capsules (Spitzer et al., 1978; King et al., 1988) and for 3.6 mg norgestomet silicone implants as well (Favero, 1992). In the current trial implants bearing 8 mg of norgestomet were 100% (18/18) effective to suppress estrus in the non-pregnant resynchronized cows. On the other hand, a 6 mg norgestomet implant only achieved 82.3% (13/17) of efficiency for the same period of time. Similar observation was made by Favero (1992) who concluded that silicone implants containing 10 mg of norgestomet and kept in situ for 9 to 12 days are effective in suppressing estrus of previously synchronized beef cows.

Table 3-4 combines behavioral trial observations with the outcomes from both the in vitro assay and the in vivo determination of norgestomet delivery. Corrected values (Figure 3-1) were employed.

Data in the Table 3-4 made it possible to determine the threshold for effective biological activity of norgestomet. The appearance of the first breakthrough estrus occurred on day 13 after implants insertion and coincided with a corrected norgestomet

release of 121 ± 8.6 μg and an expected release of 124 μg. These values are considerably higher than those observed for the two remaining breakthrough estrus (Table 3-4). At this point, considerations about the still incipient procedure of manufacturing the implants should be taken into account. For instance, a millimeter difference in the length of the small implant would cause a reduction of 4.8% in its initial surface area (from 166 mm^2 to 158 mm^2) and a 5.3% decrease in its theoretical raw norgestomet content (from 6.0 mg to 5.3 mg). In addition, cows in the behavioral trial did not have all the same size and further work is needed to clarify whether there may be a relationship between threshold dose of norgestomet and live weight of cattle.

As shown in the table 3-4 and Figure 3-1 breakthrough estrus took place when predicted daily release of norgestomet decayed to 124 μg (13th day) or below. Moreover, a expected release of 137 μg sufficed to keep cows implanted with 6 mg capsules from estrus. Such a result was further reinforced by the fact that no cows receiving 8 mg norgestomet implants showed estrus within the 16 day period and the lowest secretion expected would take place on the 16th day since insertion and would be 136 μg. Therefore, it can be established that 136 μg of norgestomet secreted daily from implants will suppress estrus behavior in cattle. This conclusion is coherent with the actually measured and posteriorly corrected amount of steroid delivered from the 6 mg norgestomet implant because even when norgestomet was erratically released on day 11, estrus was not manifested and the secretion was 141 μg . On the

other hand, as actual secretion reached a daily value as low as 121 μg (Figure 3-1 and Table 3-4) breakthrough estrus was displayed.

CONCLUSIONS

The secretion of norgestomet from polydimethylsiloxane implants differed according to the initial load impregnated and initial surface area of the cylinder. Implants containing 8 mg of norgestomet were capable to effectively suppress estrus in postpartum beef cows submitted to estrus resynchronization. Implants with 6 mg of norgestomet were not efficient in inhibiting estrus for longer than 12 days in situ. Estrus was suppressed when polydimethylsiloxane implants were expected to have a daily release of norgestomet of at least 136 μg.

FIGURE 3-1. PREDICTED DAILY RELEASE (μg) OF NORGESTOMET FROM THE IMPLANTS.

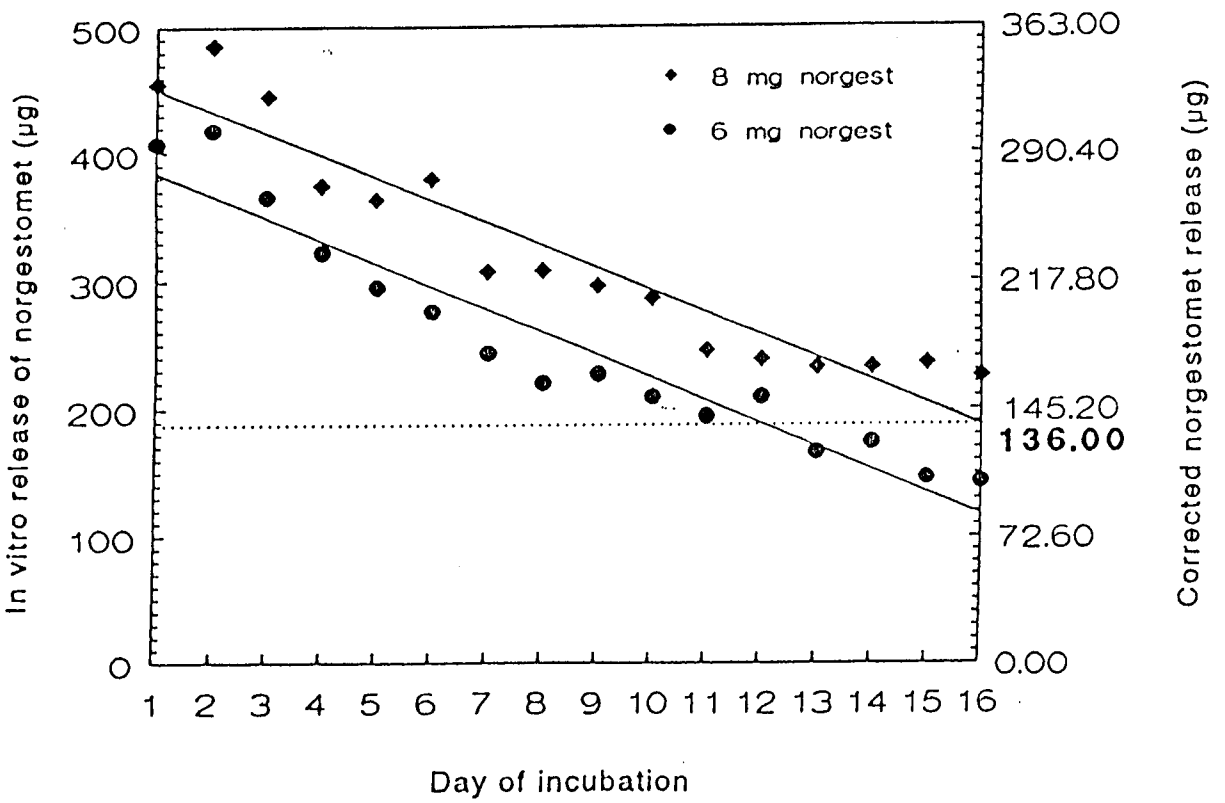

FIGURE 3-2. CORRECTED CUMULATIVE SECRETION OF NORGESTOMET (μg) FROM SILICONE IMPLANTS OVER A 16 DAY LONG IN SITU PERIOD.

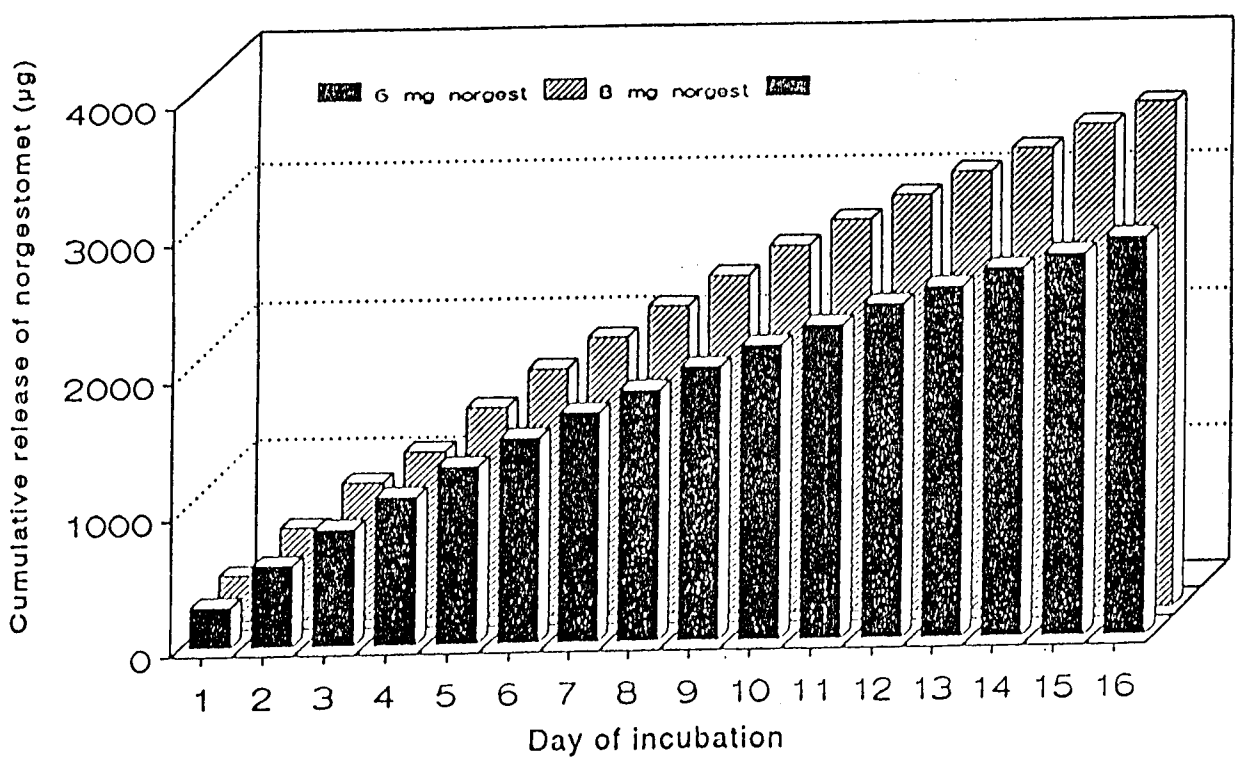

TABLE 3-2. EQUATIONS FOR THE PREDICTION OF THE DAILY RELEASE OF NORGESTOMET FROM POLYDIMETHYLSILOXANE IMPLANTS.

Implant load	Linear Equation[1]	r	R^2
6 mg	Y = -17.8059 X + 402.2250	- 0.9597	0.9210**
8 mg	Y = -17.5603 X + 468.3250	- 0.9350	0.9082**

[1] Y = mass of norgestomet released and X = day of incubation

** p<.01

TABLE 3-3. TOTAL CONTENT (mg), *IN VIVO* AND *IN VITRO* SECRETION (mg) OF NORGESTOMET FROM SILICONE IMPLANTS ACCORDING TO INITIAL STEROID LOAD.

Norgestomet content (mg)	Type of implant			
	6 mg[1]		8 mg[2]	
	Mean ± sd	CV(%)	Mean ± sd	CV(%)
Total[3]	6.21±0.09	1.4	8.33±0.16	3.8
In vivo recuperation[4]	3.17±0.10	3.1	4.57±0.33	7.2
In vivo secretion[5]	3.04		3.76	
In vitro recuperation[6]	2.02±0.11	5.4	3.15±0.24	7.2
In vitro secretion[7]	4.19		5.18	
In vitro secretion[8]	4.01		5.46	
In vitro/*In vivo* ratio[9]	4.19/3.04 = 1.378		5.18/3.76 = 1.378	

[1] Implant designed to contain 6 mg of norgestomet.
[2] Implant designed to contain 8 mg of norgestomet.
[3] After complete extraction with methanol at 37 °C for 6 days.
[4] Content remaining in the implant after left 16 days *in situ*.
[5] Total content minus *in vivo* recuperation.
[6] Content remaining in the implant after a 16 days *in vivo* assay.
[7] Total content minus *in vitro* recuperation.
[8] Cumulative *in vitro* secretion observed after a 16 *in vitro* assay.
[9] Ratio used to compute the correction factor for *in vivo* kinetics.

TABLE 3-4. DETECTION OF BREAKTHROUGH ESTRUS ACCORDING TO DAY OF RESYNCHRONIZATION AND CORRECTED AMOUNTS OF NORGESTOMET RELEASED FROM THE POLYDIMETHYLSILOXANE IMPLANT LOADED WITH 6 mg OF NORGESTOMET.

Cow ID No.	Breakthrough estrus	Norgestomet release (μg)	
		Actual	Predicted
7073	13th	121	124
7435	15th	106	98
5205	16th	104	85

CHAPTER IV.

THE EFFICACY OF SIXTEEN DAY NORGESTOMET POLYDIMETHYLSILOXANE IMPLANTS ADMINISTERED FIVE DAYS AFTER AN ARTIFICIAL INSEMINATION ON CALVING RATE

SUMMARY

An investigation was conducted aiming to evaluate if silicone implants impregnated with different amounts of norgestomet were efficacious to resynchronize the return to estrus in previously synchronized inseminated but non-pregnant postpartum beef cows, thus allowing for a second timed-artificial insemination. Fifty-nine postpartum beef cows were synchronized by means of Syncro-Mate B (SMB) program which consisted of an injection of 3 mg norgestomet and 5 mg estradiol valerate associated with the subcutaneous insertion of a 6 mg norgestomet implant in the ear. The implant remained in situ for 9 days and cows were artificially inseminated 48 after implant removal. A 25 mg injection of $PGF_{2\alpha}$ had been given 5 days before SMB to 30 cows. An additional 6 mg or a 8 mg norgestomet silicone implant was given respectively to 29 and 30 cows five days after service. Implants were withdrawn 16 days later and a second artificial insemination was performed 48 hours after implant removal only in cows with low serum progesterone (<1.5 ng/ml) at implant removal (n=34) or showing heat after norgestomet capsule have been pulled out (n=1). $PGF_{2\alpha}$ pretreatment did not affect (p>.05) calving rate to the second artificial insemination and the overall cumulative, i.e. initial plus second

services, calving rate was 57.1%. However, the negative effects of $PGF_{2\alpha}$ on the fertility of the initial artificial insemination led cumulative calving rate be non-significantly ($p>.75$) lower (53.6%) than that observed to cows not receiving $PGF_{2\alpha}$ (60.7%). Similarly, the calving rate to the second artificial insemination was significantly higher ($p<.05$) than the calving rate to the initial breeding only for the group pretreated with $PGF_{2\alpha}$ (52.9% vs. 21.4%), which corroborates the conclusion about the detrimental effects of $PGF_{2\alpha}$ on the fertility to the initial service. A greater load of norgestomet (8 mg) effectively suppressed estrus for 16 days and resulted in a higher ($p>.75$) calving rate (55.5%) than that observed for cows reimplanted with 6 mg capsules (41.2%). This difference was the result of breakthrough estrus (17.6%) seen in the 6 mg norgestomet implanted females. The overall accuracy of serum progesterone analysis to define which cows were to be rebred was 87.9%. It is possible that the cutoff point employed should be elevated to enhance the number of correct diagnoses. This should increase the number of cows destined to be reinseminated and the likelihood of a higher pregnancy rate to a second timed-artificial insemination. In summary, the use of norgestomet silicone implants in resynchronizing protocols made it possible to artificially inseminate on different occasions without the need of estrus detection. The number of calves born to artificial insemination exceeded 50.0% and reached 60.7% for cows not pretreated with $PGF_{2\alpha}$ in the same calving season.

INTRODUCTION

Synchronization of estrus facilitates the use of genetically superior sires through artificial insemination and may enhance reproductive performance by allowing for a shortened breeding season and a shortened calving season (Odde, 1990). Mass-mating or artificial insemination by appointment is utilized after synchronized estrus to reduce the extensive labor and management involved with estrus detection. However, conception rates to the single-timed artificial insemination are variable and frequently low. It is well known that fertility to the first postpartum estrus is lower than subsequent estrus (Pleasants and Barton, 1992) due to abnormal luteal phases (Garverick and Smith, 1986) and embryonic losses (Niswender and Nett, 1988; Van Cleeff et al., 1991). In this scenario, superior genetics from proven bulls usually reach less than half of beef cattle that use estrus synchronization and artificial insemination solely once in the breeding season. The wide spread of genes of interest and their impact on the genetic merit of a particular population are thus limited.

Efforts to augment conception rates to timed artificial insemination after estrus synchronization have been made. The response in fertility after progesterone or progestogen postinsemination supplementation has been variable. Van Cleeff et al. (1991) reported similar conception rates for control (53.6%) and treated heifers that had received a progesterone release intravaginal device (PRID) on day 7 through day 13 post service

(57.9%). Similar findings were described by Stevenson and Mee (1991). Silicone implants with 6 mg of norgestomet given 9 days after artificial insemination did not enhance calving rate of beef heifers (Domatob, 1993). On the other hand, implants containing 3 mg of norgestomet administered at the time of embryo transfer, i.e. day 7 after breeding, improved pregnancy rates of recipient cows (Broadbent et al., 1992). In addition, treatment with norgestomet implants on day 9 or 12 through day 21 increased calving rates to the timed artificial insemination of beef heifers (Favero et al., 1993). Nonetheless, the magnitude of reported improvements is low and frequently non-significant. In this case, females that do not conceive to the timed-artificial insemination need to be heat checked if artificial insemination is to be employed for the subsequent matings (Favero et al., 1993). Such a situation encourages producers to use conventional reproductive management rather than adopt estrus synchronization and artificial insemination (Kaim et al., 1990).

A second opportunity for a timed-insemination could circumvent the limitations previously mentioned. As a matter of fact, a repetitive synchronizing program based on two eight-day treatment with norgestomet enhanced pregnancy rate in anestrous heifers within a 24-day breeding season (Ghallab et al., 1984). Van Cleeff et al. (1991) verified that the resynchronization of estrus by means of administration of PRID on day 17 and kept in place until day 22 promoted a concentration of estrus and improved the conception rates of both previously inseminated and non-inseminated

nonpregnant heifers. In addition, treatments with different amounts of norgestomet on day 9 or day 12 through day 21 after the initial service increased calving rate of beef heifers and efficiently synchronized the return to estrus of non pregnant treated heifers (Favero et al., 1993). It was concluded that administration of norgestomet at mid luteal phase of estrous cycle had no detrimental effects on the fertility of prior or subsequent estrus and caused the nonpregnant females to exhibit estrus in a more synchronized manner (Favero et al., 1993).

The effectiveness of norgestomet implants to suppress estrus depends on the amount of steroid impregnated (Favero 1992), period of time in situ (King et al., 1988; Coy and Garcia-Winder, 1991; Favero et al., 1993), type of polymer and proportion of cross-linkage used for manufacturing the implant (Spitzer et al., 1978; Favero, 1992) and surface area (Kesler, 1989; Favero, 1992).

The purpose of this study was to compare the effects of norgestomet load impregnated onto polydimethylsiloxane (silicone) implants, which were inserted five days after the initial artificial insemination and kept in situ for 16 days on the calving rate to a second timed-artificial insemination in postpartum beef cows.

MATERIAL AND METHODS

Fifty-nine suckled postpartum Angus and mixed breed beef cows had been previously synchronized by means of an intramuscular injection of norgestomet (3.0 mg) and valerate estradiol (5.0 mg)

in a sesame oil and benzyl alcohol (10%). At the same time a silastic implant containing 6.0 mg of norgestomet was subcutaneously inserted into the convex surface of the ear. The implant was removed 9 days after its insertion and the cows were artificially inseminated approximately 48 hours after implant withdrawal. Thirty cows received an injection of 25 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as dinoprost tromethamine five days before the synchronizing treatment. All cows were randomly assigned to two experimental lots and received either a 6 mg (n=29) or a 8 mg (n=30) norgestomet silicone implant. Implants were subcutaneously inserted into the ear on day 5 after the initial artificial insemination and remained in situ for 16 days. Blood samples were obtained at the same time of implants removal and serum was obtained and processed according to technique described by Wiseman et al. (1982). Progesterone concentration was determined in the serum through a validated enzyme linked immunosorbant assay (ELISA; Kesler et al., 1990). Cows which had progesterone concentration lower than 1.5 ng/ml were artificially inseminated approximately 48 hours after the implants had been withdrawn. In addition, cows in heat by the time of the timed insemination were inseminated regardless of progesterone concentration two days before.

Concentration of 1.5 ng/ml or above at the time of explantation was considered to indicate pregnancy to the first timed-artificial insemination (Favero et al., 1993).

Only calvings placed on 283 ± 11 days from the date of the second artificial insemination were considered to determine calving rates according to implant administered.

The Chi-square test was used to determine differences in calving rate among treatment groups. Analysis of covariance was also performed utilizing a linear model where main effects assessed were pretreatment with $PGF_{2\alpha}$, cyclicity status at the first synchronization, progesterone level at initial implantation, type of implant at resynchronization and the covariate used was days postpartum to initiation of treatment. Interactions among factors were also assessed (Steel and Torrie, 1980). Student's t test was employed to compare the means of progesterone concentration between correct and erroneous diagnoses of pregnancy. All analysis were run using the SAS program (SAS User's guide, 1985).

RESULTS AND DISCUSSION

During the course of the experiment two cows lost their implants, also one cow was missing for the appointed implant removal and blood collection. As a result, the number of cows per group on which data were evaluated became as follows: 6 mg implant: n=28 and 8 mg norgestomet implant: n=28.

Thirty-four cows had serum progesterone concentrations lower than 1.5 ng/ml at implant removal. One cow that had shown progesterone concentration ≥ 1.5 ng/ml at implant withdrawal was in heat at the time of the second service and was artificially inseminated, as well. Therefore, 17 and 18 cows respectively from

6 mg and 8 mg norgestomet implant group were submitted to a second artificial insemination by appointment.

The Table 4-1. shows calving rates obtained after each synchronizing procedure and the combined results.

There was no control group to assess the effect of resynchronization with norgestomet implant on the fertility to the initial artificial insemination. However, as showed in the chapter II, the effects of location and its interaction with treatment were non-significant (p=0.41), which makes possible to look at the initially inseminated non-resynchronized cows at DSAC (Table 2-4) as a contemporary reference herd rather than a control group. From this approach, apparently no significant improvements in fertility to the first service were achieved by progestogen supplementation started at the beginning of the luteal phase, i.e. day 5 of estrous cycle. Such a supposition is supported by findings of Favero (1992) where no effects of norgestomet administered at the mid-luteal phase and removed 21 days after the previous service were observed on the calving rate to the first artificial insemination. Domatob (1993) reported concurring results after having used a similar resynchronizing program for yearling beef heifers. In addition, progesterone supplementation given as PRID did not significantly improve conception rate to the previous service (Stevenson and Mee, 1991; Van Cleeff et al., 1991).

The progesterone/progestogen postinsemination supplementation had been considered an alternative to enhance luteal function and thereby ensure early embryonic survival and development (Niswender

and Nett, 1988). This hypothesis was somewhat proven by the higher pregnancy rates after embryo transfer on day 7 of the cycle of recipient cows reported by Broadbent et al. (1992). In the present trial, the decision to commence the supplementation with norgestomet on day 5 of the estrous cycle was based on findings of Garverick and Smith (1986) and made in an attempt to affect a stage where corpora lutea anticipated to be short-lived had undergone development, but had not begun to regress. However, Butcher et al. (1992) concluded that neither 25 mg of norgestomet implant nor 100 mg of progesterone given until 35 days postmating were sufficient to maintain pregnancy from transferred embryos after the first postpartum ovulation in cows with short or normal duration of luteal phase. It was also observed in the same study that norgestomet prevented ovulation, suppressed estrus but follicular growth and estradiol secretion continued to take place. Therefore, it appears that the dysfunctional corpus luteum is not the only major cause of low fertility at the first postpartum estrus. Furthermore, embryonic mortality in postpartum beef cows as low as 3.5% has been reported (Blasco and Revilla, 1992). This means that the target population for the beneficial action of progestational postbreeding therapy may represent only a small fraction of the entire herd and improvement on the conception rate to the previous service can be unnoticeable when computed to the herd as a whole.

The overall fertility to the initial artificial insemination was not significantly different ($p>.05$) from the calving rate to the second regardless the type of implant inserted (Table 4-1).

Nonetheless, the proportion of pregnant cows to the second service (17/35 = 48.6%) was non-significantly higher (p>.05) than that for the initial artificial insemination (15/56 = 26.8%). The detrimental effect of pretreatment with $PGF_{2\alpha}$ may partially explain that difference. In addition, it is assumed that a greater proportion of cyclic cows were exposed to the second artificial insemination because cows were averaging 91.51 ± 12.00 days postpartum at the second service compared to 68.42 ± 15.20 days at the initial artificial insemination. Furthermore, Syncro-Mate B treatment has shown to be capable to induce ovarian activity in anestrous cows (Hixon et al., 1981) and as discussed in the chapter II, calving rate tended (p.>50) to be higher for cyclic than for anestrous cows (Table 2-4.) and degree of cyclicity was directly related with days postpartum.

No detrimental effect on pregnancy rate following removal of implants on the 16th day of insertion was herein observed. Broadbent et al. (1992) reached to same conclusion after using 3 mg norgestomet implant from day 7 through day 19 in embryo recipient beef cows. Likewise, Favero et al. (1993) verified that a 9 to 12 day norgestomet treatment during a specific stage of the estrous cycle does not diminish the fertility at the detected estrus. This set of similar conclusions is anchored by the findings of Coy and Garcia-Winder (1991) who verified that a 6 mg norgestomet implant maintained <u>in situ</u> for a period of 9 or 18 days did not alter corpus luteum life span and ovaries showed to be responsive to

gonadotrophins with formation of a normal luteal tissue, identical to that observed during the estrous cycle of the cow.

The overall calving rate achieved after both timed-artificial inseminations was lower (Table 4-1.) than rates previously reported for beef cows (69%) and beef heifers (73%) by Favero (1992). Nonetheless, the cumulative conception rates of cows that not received $PGF_{2\alpha}$ pretreatment is comparable (60.7%) to ranges found by Favero (1992) then implying that the poor overall results herein reported are caused by the detrimental effects of the pretreatment with $PGF_{2\alpha}$ on the fertility to the first service (21.4%).

Table 4-2. summarizes the effects of the type of second implant on the calving rate to the second artificial insemination.

8 mg norgestomet implants tended ($p>.75$) to improve fertility at the second-timed artificial insemination for both $PGF_{2\alpha}$-pretreated (55.5%) and non $PGF_{2\alpha}$-pretreated (55.5%) cows compared to 50.0% and 33.3% for cows reimplanted with the 6 mg norgestomet implant and that respectively received and did not receive $PGF_{2\alpha}$ injection prior to the initial synchronization. It is reasonable to infer that no detrimental carry over effects of pretreatment with $PGF_{2\alpha}$ took place on fertility to the second service.

It must be mentioned that the occurrence of breakthrough estrus in cows receiving 6 mg norgestomet implants (3/17 = 17.6%) was significantly ($p<.05$) higher than that observed for cows implanted with 8 mg norgestomet cylinders (0/18 = 0.0%). Furthermore, as expected none of cows displaying estrus while implant was <u>in situ</u> became pregnant to the second artificial

insemination. Therefore, the marginal suppression of estrus provided by the 6 mg norgestomet implant led to a non-significant ($p>.75$) reduction in the calving rate to the second timed-artificial insemination and mistimed service is to be implicated in the failure of fertilization. This conclusion was drawn from the fact that estrus in inseminated non pregnant cattle submitted to progestational supplementation has been consistently shown to be displayed in a very concentrate fashion (Van Cleeff et al., 1991; Favero, 1992; Favero et al., 1993), which may play a role in reducing the number of unsuccessful fertilizations due to mistiming.

Suppression of estrus with 6 mg norgestomet implants for longer than 9 days has not achieved 100% of efficiency. Spitzer et al. (1978) demonstrated that hydron implants with crosslinkage of 4% or more and retained in situ for 21 days in cattle led to the appearance of breakthrough estrus within a range of 3 and 65%. An alternative synchronizing program where a 6 mg norgestomet implant is maintained 14 days in situ was devised by King et al. (1988), who reported some frequency of estrus signs while implants were still in place. Favero (1992) also reported breakthrough estrus in cows (less than 1%) and Favero et al. (1993) reported 5% in heifers (1/20) treated with 3.6 mg norgestomet-silicone implants for 9 or 12 days after an initial artificial insemination.

The silicone implant containing 8 mg of norgestomet efficiently suppressed estrus in beef cows for 16 days and promoted a satisfactory calving rate to the subsequent insemination

(Table 4-2). Likewise, as long as cows were kept from standing estrus with 6 mg norgestomet implants the fertility was comparable [7/(17-3) = 50.0%] to that observed for cows implanted with 8 mg cylinders (10/18 = 55.5%). Therefore, it appears that the phenomena leading to ovulation are strictly related to estrus suppression in norgestomet resynchronized cows. In addition, the pooled (6 mg and 8 mg implants) calving response to resynchronization would have become 53.1% [10+7/(18+17-3)] for cows which estrus was efficiently suppressed after the first service. Such an occurrence might have been caused by a more tight synchrony between LH surge, ovulation subsequent to implant removal and the time of the second artificial insemination.

The accuracy of progesterone concentration measurements to predict pregnancy and determine cows to be bred at a second synchronized service is compiled in the Table 4-3.

Favero (1992) proved that mass insemination after resynchronization procedure results in a non-significant decrease in the calving rate to the initial artificial insemination and the use of progesterone peripheral determination improved the proportion of females that became pregnant to the first two services.

Accuracy in determining pregnancy status based on serum progesterone levels on day 21 after initial artificial insemination was 88.1% (Table 4-3), which is in agreement with the value found by Favero (1992), who reported 87.7% using the same cutoff point

(1.5 ng/ml) and assayed the samples by the same immuno technique it was performed in this study.

Accuracy observed for cows considered pregnant (high progesterone) and that did not calve due to the initial breeding was unexpectedly low (15/22 = 68.2%) and again, $PGF_{2\alpha}$ pretreated cows behaved differently ($p>.05$) from the other cows. Nevertheless, four of five cows pretreated with $PGF_{2\alpha}$ and mistakenly determined pregnant were not cycling at the time of $PGF_{2\alpha}$ injection and it is unlikely that the prostaglandin affected the subsequent luteal phase of those females.

Cows accurately determined as pregnant showed a significantly higher ($p<.05$) serum concentration of progesterone (9.53 ± 1.83 ng/ml) 21 days after breeding than cows considered pregnant and that failed to calve to the initial artificial insemination (6.02 ± 4.16 ng/ml). Moreover, the lowest concentration of progesterone observed for accurately determined pregnant cows (n=15) was 7.17 ng/ml, in contrast to four cows erroneously taken as pregnant and that had concentrations lower than 4.00 ng/ml. Therefore, pregnancy seemed to offer a stronger stimulus for progesterone secretion by the corpus luteum, which agreed with previous findings of Domatob (1993). In this case, an enhanced luteal function related to gestation represents an important discriminator between spurious and verum corpora lutea. As a result, accuracy of pregnancy determination by means of serum progesterone measurements can be improved with the use of a greater cutoff point than that applied to determine cyclicity status (≥ 1.5 ng/ml).

Table 4-4 shows the hypothetical increase in serum progesterone cut-off points and their effect on several parameters related to pregnancy diagnosis in cows. It can be verified that as cut-off increases up to 4.0 ng/ml, the specificity, accuracy and positive predictive value of the assay also increase. As a result, fewer false positive diagnosis would be made and no increase in the number of false-negatives would happen.

Improvements in these parameters affect the number of cows destined to be re-inseminated after the removal of the second implant and consequently increase the likelihood of obtaining a higher combined calving rate to the two first timed-breedings.

CONCLUSIONS

The use of norgestomet silicone implants inserted five days after an initial breeding did not have negative effects on previous or subsequent artificial insemination. 8 mg norgestomet implant efficiently suppressed estrus for a period of 16 days and resulted in an increased ($p>.75$) calving rate to the second artificial insemination if compared to a 6 mg norgestomet implant. On the other hand, 17.6% of cows implanted with 6 mg norgestomet implant exhibited estrus while the steroid capsule was still in place and fertility to the second timed-service was reduced due to mistimed artificial insemination. Accuracy of pregnancy diagnosis on the 21th day after initial breeding may be improved by elevating the cutoff point (1.5 ng/ml). The average and the lowest concentrations of serum progesterone obtained for accurately

determined pregnant cows were far above the respective values observed for erroneously diagnosed as pregnant cows. In summary, resynchronization protocols herein described effectively permitted the use of timed-breeding in two distinct occasions and resulted in a higher number of cows calving to artificial insemination.

TABLE 4-1. EFFECT OF THE RESYNCHRONIZATION OF ESTRUS ON CALVING RATES OF POSTPARTUM BEEF COWS PREVIOUSLY TREATED WITH SYNCRO-MATE B AND ASSOCIATED OR NOT WITH $PGF_{2\alpha}$ PRE-ADMINISTRATION.

	Calving rate							
		1st AI			2nd AI		Cumulative	
	N[1]	n	%	N	n	%	n	%
No-$PGF_{2\alpha}$	28	09	32.1[a]	18	08	44.4[a]	17	60.7
$PGF_{2\alpha}$	28	06	21.4[b]	17	09	52.9[a]	15	53.6
Overall	56	15	26.8[a]	35	17	48.6[a]	32	57.1

[1] Two cows lost their implants and one cow was missing at the time of implant removal (59-3=56).

[a,b] Figures with different superscripts within the same row differ ($p<.05$).

TABLE 4-2. EFFECT OF THE TYPE OF THE 2nd IMPLANT ON THE CALVING RATE TO THE 2nd ARTIFICIAL INSEMINATION OF POSTPARTUM BEEF COWS PREVIOUSLY SUBMITTED TO THE SYNCRO-MATE B PROGRAM.

Implant[1]	Treatment								
	No-$PGF_{2\alpha}$			$PGF_{2\alpha}$			Overall		
	N	Calving		N	Calving		N	Calving	
		n	%		n	%		n	%
6 mg	8	3	33.3	8	4	50.0	16	7	43.7
8 mg	10	5	50.0	9	5	55.5	19	10	52.6
Total	18	8	44.4	17	9	52.9	35	17	48.6

[1] No statistically significant ($p>.05$) differences were found.

TABLE 4-3. ACCURACY[1] OF SERUM PROGESTERONE (P_4) ANALYSIS[2] TO DETERMINE THE NUMBER[3] OF COWS TO BE RE-INSEMINATE AFTER SYNCHRONIZATION WITH SYNCRO-MATE B ASSOCIATED OR NOT WITH $PGF_{2\alpha}$ PRE- TREATMENT AND SUBMITTED TO RESYNCHRONIZATION WITH NORGESTOMET IMPLANT[4].

P_4 level[5]	Diagnosis[6]				Accuracy (%)[7]		
	Pregnant		Open				
	No $PGF_{2\alpha}$	$PGF_{2\alpha}$	No $PGF_{2\alpha}$	$PGF_{2\alpha}$	No $PGF_{2\alpha}$	$PGF_{2\alpha}$	Combined
High	09	06	02	05	81.8 (9/11)	54.5 (6/11)	68.2 (15/22)
Low	0	0	17	19	100.0 (17/17)	100.0 (19/19)	100.0 (36/36)
Overall					92.8 (26/28)	83.3 (25/30)	87.9 (51/58)

[1] Relation between number of correct diagnoses and total number of diagnoses made.

[2] Serum determination through a validated ELISA.

[3] One cow was missing for blood collection (59-1=58).

[4] All cows were submitted to the traditional SM-B program associated or not with a 25 mg $PGF_{2\alpha}$ injection five days before implant insertion. The second norgestomet implant was administered on the fifth day subsequent to the initial breeding and maintained *in situ* for 16 days.

[5] High level was considered to be concentrations of $\geq$ 1.5 ng/ml and low level was concentrations of $<$ 1.5 ng/ml.

[6] Cows with high level of P_4 at removal of the 2nd implant were considered pregnant to the initial artificial insemination (on the day 21 since breeding).

[7] Number of animals is in parentheses.

TABLE 4-4. EFFECT OF VARYING SERUM PROGESTERONE CONCENTRATION CUT-OFF POINTS ON ACCURACY, SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE, AND NEGATIVE PREDICTIVE VALUE OF DAY 21 SERUM PROGESTERONE CONCENTRATIONS IN ESTIMATING PREGNANCY STATUS OF SYNCHRONIZED BEEF COWS.

P_4 cutoff (ng/ml)[1]	Sensitivity[2]	Specificity[3]	Accuracy[4]	Predictive value	
				Pos[5]	Neg[6]
1.0	100.0%	79.1%	86.4%	65.2%	100.0%
1.5	100.0%	81.4%	87.9%	68.2%	100.0%
2.0	100.0%	83.7%	91.4%	75.0%	100.0%
2.5	100.0%	88.4%	91.4%	75.0%	100.0%
3.0	100.0%	88.4%	91.4%	75.0%	100.0%
3.5	100.0%	88.4%	93.1%	78.9%	100.0%
4.0	100.0%	90.7%	94.8%	83.3%	100.0%
No data from 4.5 to 7.0 ng/ml					
7.5	86.7%	93.0%	91.4%	81.2%	95.3%
8.0	66.7%	93.0%	87.9%	78.6%	91.1%

[1] Females with day 21 serum progesterone concentration ([P_4]) less than this level were classified as non pregnant and females with day 21 serum progesterone concentrations greater than this level were considered pregnant.

[2] Number of pregnant females having [P_4] greater than the cutoff point divided by the total number of pregnant females.

[3] Number of non-pregnant females having [P_4] less than the cutoff point divided by the total number of non-pregnant females.

[4] The proportion of all test results, both pregnant and non-pregnant, that were correct.

[5] Number of pregnant females divided by the total number of females with [P_4] greater than the cutoff level (1 - false positives).

[6] The proportion of females that indicated non-pregnancy, that were non-pregnant (1 - false negatives).

CHAPTER V

GENERAL INTERPRETATIVE DISCUSSION

The present study addressed techniques to enhance fertility to artificial insemination in postpartum suckled beef cows submitted to a commercially available estrus synchronization program.

The basic intent in synchronizing estrus and induced ovulation in beef cattle is to minimize labor involved with estrus detection and artificial insemination (Odde, 1990). Nonetheless, the biological response of postpartum suckled beef cows has been only marginal to the available protocols (Troxel et al., 1983; Odde, 1990). As a consequence, frequently economic considerations discard the use of artificial insemination in beef operations due to the unfavorable cost-benefit ratio (Cooper, 1978; Odde, 1990). However, the elevated cost of maintenance of postpartum beef cows (Favero, 1992), the need to quickly meet the market demands for beef cattle with desired carcass characteristics (Neumann and Lusby, 1986; Savell et al., 1989) and the abundance of information available on estrous cycle regulation (Odde, 1990; Larson and Ball, 1992) keeps alive the search for cost effective and biologically efficient methods to synchronize and induce ovulation in suckled beef dams.

The present work was based on a series of three distinct, although related, experiments. The main objectives were to verify the role played by the stage of the estrous cycle at the beginning of the Syncro-Mate B (SMB) program on the calving rate and to determine the minimum secretion of a progestogen (norgestomet) from

a polydimethylsiloxane (silicone) implant capable to efficiently suppress estrus in cows. Relevant appended information was also gathered as the main goals of this study were pursued.

The use of silicone implants in substitution for hydron-based polymers had been demonstrated to enhance fertility (p.>05) of beef cows submitted to SMB (Kesler and Favero, 1989, Favero, 1992). In this study only silicone implants were employed and their well-known biocompatibility (Dziuk and Cook, 1966, Sun et al., 1986) allowed for a lower implant loss rate (4+2/270+59 = 1.82%) than that frequently reported for hydron capsules (Spitzer et al., 1978; Broadbent et al., 1992).

It has been shown that SMB is able to induce cyclicity in anestrous bovine females (Miksch et al., 1978; Brown et al., 1988). Nonetheless, fertility of cyclic cows is generally higher than that verified for anestrous females (Miksch et al., 1978; Troxel et al., 1983; Whittier et al., 1986). In order to asses the affect of the stage of estrous cycle at SMB on fertility, the experiment was designed with cows averaging more than sixty days postpartum, which would ensure a greater proportion of cyclic cows at implant insertion (70.4% of cows were cyclic). According to the experimental sampling procedure used it was possible to subdivide the estrous into four segments (d 1 to d 5; d 6 to d 10; d 11 to d 17 and d 18 to d 0) and cows synchronized during the first half of the cycle showed significantly (p>.05) lower calving rate than those on the second half of the estrous cycle.

Brink and Kiracofe (1988) hypothesized a better luteolytic action of the estradiol valerate if given early in the estrous cycle and reported higher fertility for cows synchronized during the first half of the cycle. Such a hypothesis was proved to be trustful since the fact that luteal regression was hastened when SMB was administered prior to day 11 of the cycle (Kesler et al., 1984; Kesler and Favero, 1993). On the other hand, several studies found opposite results (Pratt et al., 1991; Fanning et al., 1992; Burns et al., 1993) where consistently a large number of cows treated early in the cycle maintained secretory luteal tissue upon implant removal. This event would indicate the inefficaciousness of SMB to regress existent or prevent development of corpus luteum of females treated within metestrus (Fanning et al., 1992; Burns et al., 1993).

Cows pre-treated with $PGF_{2\alpha}$ showed a significant ($p<.05$) lower fertility (23.7%) than control cows (33.1%). Surprisingly, when $PGF_{2\alpha}$ effectively promoted luteal regression (65.5%) calving rate was severely depressed (7.9%), in opposition to cows receiving prostaglandin and that did not undergo luteolysis (50.0%).

Response to $PGF_{2\alpha}$ is variable among cows (Garcia-Winder and Gallegos-Sanchez, 1991) which leads to a relatively asynchronous proestrus (Kerr et al., 1991). In addition, it has been determined that the most dramatic endocrine changes throughout the estrous cycle take place within its follicular phase (Hafs et al., 1974; Gore-Langton and Armstrong, 1988). Therefore, asynchronies as short as 24 hours in that period may elicit completely opposite

response to estradiol valerate (Bo et al., 1993), which would ultimately represent either hastened or delayed emergence of follicular growth. Aberrant follicular development may lead to the formation of abnormal corpus luteum because granulosal and thecal cells are the precursors of luteal cells (Niswender and Nett, 1988; Sanchez et al., 1993).

Cows with no corpus luteum due to the lytic action of the exogenous prostaglandin showed the lowest fertility (7.9%) among cyclic cows. These results are not in complete disagreement with recent findings of Sanchez et al. (1993), where cows bearing corpus luteum had higher fertility if compared to cows led to luteolysis by the time of norgestomet implant insertion. However, the experiment design used by Sanchez et al. (1993) incorporated a $PGF_{2\alpha}$ injection on day 17 of luteal phase with concomitant withdrawal of implants. This group of cows had the advantage of the synergic luteolytic effects of exogenous $PGF_{2\alpha}$ (Melampy and Anderson, 1968) and endogenous luteolysin (Niswender and Nett, 1988). Therefore, the comparison between groups of the effect of presence or absence of corpus luteum at implantation became compromised. The same study showed that cows with corpora lutea had a significantly lower estrogens level when implants were in place, whereas cows submitted to luteolysis at the time of implant insertion did not benefit from the sudden decay in progesterone concentrations coupled to a rise in estrogens (Hafs et al., 1974) and eventual release of pre-ovulatory levels of luteinizing hormone (Hafs et al., 1974; Mutiga et al., 1993). Moreover, data gathered in the present study

indicated that no significant differences ($p>.05$) in calving rate take place between cows secreting luteal levels ($\geq$ 1.5 ng/ml) of progesterone (30.4%) and cows under no influence of progesterone (27.2%) regardless of being cycling or in anestrous.

In conclusion, fertility of cattle synchronized with norgestomet-estradiol valerate combinations is affected by the stage of estrous cycle. Cows treated during the second half of the cycle are expected to perform better than both anestrous females and cows on the first half of the cycle.

Among several attempts to improve fertility to artificial insemination by appointment, resynchronization appears to be promising (Favero, 1992; Domatob, 1993; Favero et al., 1993). Actually, the labor is reduced and superior genetics through artificial insemination reaches a greater proportion of beef dams.

The low effectiveness of steroid sustained release devices (implants, PRID, etc.) for exposures longer than 9 days represents a hindrance to resynchronization protocols. The impregnation of silicone implants with higher doses of norgestomet than usually used (6mg) has been effective to suppress estrus until 12 days of insertion (Favero et al, 1993). However, available information is scarce to determine the minimal, and thus most cost effective, amount of norgestomet required to efficiently inhibit estrus in cattle.

In the present study, silicone implants prepared with different loads (6 and 8 mg) of norgestomet should be able to prevent estrus displaying for a 16 day long period because the

resynchronization schedule adopted started five days after the initial service. It seemed reasonable to commence the program at that time once short lived corpora lutea would already have developed without have begun precocious regression (Garverick and Smith, 1986).

The kineto-dynamics of release differed between norgestomet loads assessed and the greater surface area occupied by the 8 mg implant appeared to be responsible for a greater daily and cumulative release of the progestogen. These results had been anticipated since previous findings in the literature (Dziuk and Cook, 1966; Kesler, 1989). In vitro systems to determine drug delivery out of silicone devices have been described (Dziuk and Cook, 1966; Karsch et al., 1973). However, in vitro secretion pattern is faster than in vivo (Kesler and Favero, 1989; Favero et al., 1990). As a result, a correction coefficient must be incorporated for a more precise computation of the amount of steroid which actually leaves the core of the silicone piece. In this light, these results furnish a reliable measure of delivery, which associated with behavioral assessment of non pregnant resynchronized cows made it possible to determine the ensured secretion level (136 μg/day) of norgestomet to effectively suppress estrus in cows.

The relevance of these findings relies upon the fact that no longer, expensive, time-consuming and susceptible to errors behavioral trials are needed to validate the estrus suppressor properties of a norgestomet-silicone implant. This is due to the

determination of a threshold of effectiveness and the description and validation of a reliable *in vitro* system for the measurement of steroid release from silicone capsules.

The resynchronization procedure employed made it possible to obtain higher proportion of calves born to artificial insemination, which agrees with previous reports (Favero, 1992). Norgestomet load did not interfere with calving rate to the second artificial insemination. However, the 6 mg implant was not capable of suppressing estrus in all cows and that led to a reduction in the overall fertility to the resynchronization protocol. Therefore, 8 mg norgestomet silicone implants are recommended for resynchronization programs based on a 16 day long period of estrus suppression.

APPENDIX

TABLE A1- SERUM PROGESTERONE CONCENTRATIONS ($[P_4]$) FOR CONTROL COWS (NO $PGF_{2\alpha}$) MAINTAINED AT URBANA FARM ACCORDING TO THE TYPE OF 2nd IMPLANT EMPLOYED.

Cow ID #	$[P_4]$ (ng/ml) date of sampling			2nd implant[1]	
				type	$[P_4]$ (ng/ml)
	04/10	04/21	04/26		
0002	-	0.38	4.27	6 mg	1.02
0018	-	1.38	8.83	P	12.74
0019	-	2.25	7.28	8 mg	8.00
0022	0.86	0.43	0.96	8 mg	0.08
173	3.00	0.01	0.08	8 mg	0.14
174	-	0.49	8.76	6 mg	0.19
0203	0.77	0.10	0.39	6 mg	7.24
0243	-	2.74	0.35	lost	14.28
0245	-	0.22	4.89	6 mg	0.50
0247	0.87	0.06	0.15	8 mg	0.01
3101	-	0.01	2.51	8 mg	9.83
4163	-	4.67	5.80	6 mg	12.49
4229	0.51	1.46	0.04	8 mg	0.27
5108	-	4.43	8.49	8 mg	0.18
5201	-	6.71	1.93	8 mg	0.28
5204	-	0.10	2.65	8 mg	0.25
5205	-	0.40	6.86	6 mg	0.39
5210	-	6.25	8.39	6 mg	0.27
5220	0.33	0.01	0.24	6 mg	0.73
6153	0.45	0.01	0.10	P	12.47
6154	-	8.32	10.04	6 mg	0.55
6158	0.01	0.43	0.11	8 mg	11.78

1 The explantation and the blood collection took place at the same time on 05/28. The implant types used were coded as follows: 8 mg; 6 mg and P = conventional 6 mg.

TABLE A1- SERUM PROGESTERONE CONCENTRATIONS ($[P_4]$) FOR CONTROL COWS (NO $PGF_{2\alpha}$) MAINTAINED AT URBANA FARM ACCORDING TO THE TYPE OF 2nd IMPLANT EMPLOYED.

Cow ID #	$[P_4]$ (ng/ml) date of sampling			2nd implant[1]	
	04/10	04/21	04/26	type	$[P_4]$ (ng/ml)
6167	-	0.40	3.79	8 mg	11.28
7037	-	7.46	6.23	6 mg	0.17
7073	-	3.13	0.99	6 mg	-
7166	-	0.01	2.62	8 mg	7.86
7413	0.01	0.08	0.16	P	0.22
7447	-	3.39	6.50	8 mg	8.70
8401	-	0.61	2.82	8 mg	0.17
8447	0.01	0.20	0.22	8 mg	10.34
9108	0.01	0.12	0.04	6 mg	11.37
9543	0.17	0.18	0.03	8 mg	0.15
9660	0.20	0.01	1.47	8 mg	11.62

1 The explantation and the blood collection took place at the same time on 05/28. The implant types used were coded as follows: 8 mg; 6 mg and P = conventional 6 mg.

TABLE A2- SERUM PROGESTERONE CONCENTRATIONS ($[P_4]$) FOR TREATED COWS ($PGF_{2\alpha}$) MAINTAINED AT URBANA FARM ACCORDING TO THE TYPE OF 2nd IMPLANT EMPLOYED.

Cow ID #	$[P_4]$ (ng/ml) date of sampling			2nd implant[1]	
	04/10	04/21	04/26	type	$[P_4]$ (ng/ml)
0005	-	5.62	0.03	8 mg	0.15
0214	0.41	0.01	0.18	6 mg	1.86
0222	-	0.01	2.09	8 mg	0.18
0594	0.87	0.19	0.11	6 mg	0.01
2294	-	3.70	2.86	lost	0.21
3040	1.56	0.01	0.46	lost	0.58
3123	-	5.67	10.21	8 mg	10.74
4251	-	0.12	4.51	6 mg	8.04
5211	-	1.48	5.96	8 mg	0.31
5221	4.28	0.21	0.84	8 mg	7.78
6151	0.48	0.01	0.16	6 mg	2.01
6188	-	0.01	2.93	6 mg	11.27
7029	0.01	0.01	0.77	P	0.01
7043	0.08	0.04	0.97	6 mg	11.16
7052	-	4.16	0.52	8 mg	0.01
7071	0.01	0.01	0.19	6 mg	0.62
7087	-	0.01	3.32	8 mg	0.39
7088	0.01	0.01	0.48	8 mg	0.50
7125	-	5.43	0.66	6 mg	0.02
7193	2.07	0.55	0.10	6 mg	7.17
7212	-	0.18	4.29	6 mg	8.63
7416	0.01	0.56	0.37	6 mg	0.39

1 The explantation and the blood collection took place at the same time on 05/28. The implant types used were coded as follows: 8 mg; 6 mg and P = conventional 6 mg.

TABLE A2- SERUM PROGESTERONE CONCENTRATIONS ($[P_4]$) FOR TREATED COWS ($PGF_{2\alpha}$) MAINTAINED AT URBANA FARM ACCORDING TO THE TYPE OF 2^{nd} IMPLANT EMPLOYED.

Cow ID #	$[P_4]$ (ng/ml) date of sampling			2^{nd} implant[1]	
	04/10	04/21	04/26	type	$[P_4]$ (ng/ml)
7435	-	4.61	5.14	6 mg	0.21
7437	0.01	0.07	0.11	P	10.23
7443	-	7.98	8.82	8 mg	0.23
8404	0.01	0.15	0.15	6 mg	0.20
8418	-	7.97	0.07	8 mg	0.01
8452	0.01	0.28	0.34	P	0.19
9503	-	3.12	0.69	8 mg	0.28
9512	0.01	0.14	0.04	8 mg	2.36
9545	0.01	0.16	0.49	6 mg	3.61
9593	0.10	0.80	0.20	6 mg	0.48
9547	0.22	0.93	0.02	6 mg	0.60

1 The explantation and the blood collection took place at the same time on 05/28. The implant types used were coded as follows: 8 mg; 6 mg and P = conventional 6 mg.

TABLE A3- CALVING DATA FOR CONTROL COWS (NO $PGF_{2\alpha}$) MAINTAINED AT URBANA FARM.

Cow ID #	Days postpartum to 1st AI	Conception	Days postpartum to conception	Calving date	Calving interval (days)
0002	71	Bull	>103	04/01	402
0018	125	1st AI	125	02/12	378
0019	86	1st AI	86	02/10	367
0022	82	2nd AI	105	03/16	387
173	74	Bull	>106	03/28	401
174	67				
0203	40	1st AI	40	02/20	327
0243	92	1st AI	92	02/17	-
0245	91	2nd AI	124	03/06	-
0247	44	2nd AI	67	03/18	360
3101	82	1st AI	82	02/10	363
4163	58	1st AI	58	02/14	339
4229	69				
5108	76				
5201	74				
5204	63	2nd AI	86	03/04	365
5205	76				
5210	79				
5220	55	2nd AI	78	03/15	368
6153	49	1st AI	49	02/15	331
6154	44				
6158	57	1st AI	57	02/09	333

TABLE A3- CALVING DATA FOR CONTROL COWS (NO $PGF_{2\alpha}$) MAINTAINED AT URBANA FARM.

Cow ID #	Days postpartum to 1st AI	Conception	Days postpartum to conception	calving date	calving interval (days)
6167	73				
7037	76	2nd AI	99	03/09	384
7073	68	Bull	>100	03/20	387
7166	79	1st AI	79	02/24	374
7413	56				
7447	72	1st AI	72	02/15	358
8401	77	2nd AI	100	03/07	383
8447	73	2nd AI	96	03/14	386
9108	54	1st AI	54	01/28	321
9543	62				
9660	81	1st AI	81	02/10	362

TABLE A4- CALVING DATA FOR TREATED COWS ($PGF_{2\alpha}$) MAINTAINED AT URBANA FARM.

Cow ID #	Days postpartum to 1st AI	Conception	Days postpartum to conception	calving date	calving interval (days)
0005	64	2nd AI	87	03/22	384
0214	45				
0222	43				
0594	71				
2294	71	Bull	>103	04/09	410
3040	73				
3123	77				
4251	76	1st AI	76	02/20	367
5211	67	Bull	>99	03/20	386
5221	74	1st AI	74	02/16	361
6151	39	Bull	>71	04/14	382
6188	86	1st AI	86	02/10	367
7029	51				
7043	58	1st AI	58	02/21	349
7052	72				
7071	46	2nd AI	69	03/10	354
7087	74	2nd AI	97	03/15	388
7088	51	2nd AI	74	03/11	360
7125	74	2nd AI	97	03/10	383
7193	71	1st AI	71	02/18	360
7212	77	1st AI	77	02/14	362
7416	73				

TABLE A4- CALVING DATA FOR TREATED COWS ($PGF_{2\alpha}$) MAINTAINED AT URBANA FARM.

Cow ID #	Days postpartum to 1st AI	Conception	Days postpartum to conception	calving	
				date	interval (days)
7435	79	Bull	>111	03/28	406
7437	47				
7443	75	2nd AI	98	03/01	375
8404	58	2nd AI	81	03/05	361
8418	86				
8452	52				
9503	80	2nd AI	103	03/06	385
9512	63				
9545	86	Bull	>118	04/01	417
9593	57	Bull	>89	03/25	380
9547	75	2nd AI	98	03/09	383

TABLE A5 - CALVING DATA AND SERUM PROGESTERONE CONCENTRATIONS ([P_4]) FOR CONTROL COWS (NO $PGF_{2\alpha}$) MAINTAINED AT DSAC, SIMPSON, IL.

Cow ID #	[P4] (ng/ml) date of sampling			Days postpartum to AI	Calving date	Calving interval (days)
	03/27	04/07	04/12			
0528	-	2.80	0.97	93		
0540	0.87	1.23	1.14	74	02/01	358
0541	-	9.10	11.57	76		
0554	-	1.02	1.64	54		
0558	0.29	0.86	1.11	68		
0596	-	11.28	0.58	94		
0616	-	10.99	0.34	78	01/31	361
0985	-	11.34	0.75	96	01/27	375
0989	0.58	1.14	0.75	53		
0992	0.84	0.09	0.87	52	01/29	334
0994	-	0.11	3.57	77		
2381	-	10.21	0.25	67	02/09	359
2398	-	9.06	9.00	84		
2490	0.15	0.22	0.01	38		
2499	0.35	0.31	0.09	66		
3115	-	6.42	0.01	41		
3495	0.47	0.35	0.01	87		
3497	-	3.24	4.39	83	01/27	362
3498	-	1.03	2.01	66		
4143	-	7.65	0.14	81	01/29	362
4156	0.18	0.07	0.05	49		
4201	-	0.04	4.00	83		
4466	0.18	0.11	0.15	87		

TABLE A5 - CALVING DATA AND SERUM PROGESTERONE CONCENTRATIONS ([P_4]) FOR CONTROL COWS (NO $PGF_{2\alpha}$) MAINTAINED AT DSAC, SIMPSON, IL.

Cow ID #	[P4] (ng/ml) date of sampling			Days postpartum to AI	Calving date	Calving interval (days)
	03/27	04/07	04/12			
4498	0.33	0.01	0.91	85		
5001	-	0.06	1.61	72		
5301	-	0.01	2.65	83		
5328	-	10.56	4.22	63		
5480	-	0.11	3.24	82		
5495	-	2.49	8.81	94	01/28	374
5497	-	0.31	2.35	89		
6491	-	0.26	2.93	46	02/06	335
7005	-	0.26	3.20	-		
7006	-	10.45	1.25	-		
7015	-	3.88	8.04	82	02/03	368
7059	-	0.15	7.15	82	01/28	362
7126	-	9.07	9.90	83		
7276	-	2.92	12.22	59		
7294	4.52	0.22	1.24	84		
8034	-	9.42	0.22	92		
8130	0.23	0.05	0.01	68		
8494	-	3.25	8.61	71	02/05	359
8498	-	1.17	7.99	85		
9001	-	9.59	6.39	85	01/28	365
9017	-	2.24	0.83	92		
9040	-	5.05	12.22	89	02/02	374
9077	-	8.43	0.60	93	01/29	374
9088	-	4.01	11.24	84		

TABLE A5 - CALVING DATA AND SERUM PROGESTERONE CONCENTRATIONS ([P_4]) FOR CONTROL COWS (NO $PGF_{2\alpha}$) MAINTAINED AT DSAC, SIMPSON, IL.

Cow ID #	[P4] (ng/ml) date of sampling			Days postpartum to AI	Calving date	Calving interval (days)
	03/28	04/08	04/13			
9094	0.74	1.18	1.28	44		
9128	-	1.50	0.35	87	02/03	373
9142	0.39	0.44	0.20	86		
9144	0.44	0.07	0.01	47		
X204	3.54	0.88	0.46	85		
X214	-	3.14	7.72	86		
X223	-	13.94	10.69	106		
X225	-	11.62	0.12	74		
X229	0.23	1.35	0.68	65		
0076	0.64	0.77	0.41	48		
0502	-	6.32	6.98	110	01/26	387
0516	-	1.85	5.85	103		
0532	-	0.29	4.90	103		
0542	-	1.07	3.41	103		
0549	-	0.08	3.47	85		
0552	-	9.73	0.97	105		
0570	-	8.31	0.25	90		
0574	-	10.13	0.45	109	01/26	386
0648	-	5.89	11.97	103		
0660	0.38	0.01	0.17	88	02/03	373
0986	0.15	0.31	0.23	58	01/29	338
0988	0.40	0.96	0.24	62		
2175	-	7.53	12.08	73	02/02	357
2281	-	2.56	0.20	85	02/02	369

TABLE A5 - CALVING DATA AND SERUM PROGESTERONE CONCENTRATIONS ([P_4]) FOR CONTROL COWS (NO $PGF_{2\alpha}$) MAINTAINED AT DSAC, SIMPSON, IL.

Cow ID #	[P4] (ng/ml) date of sampling			Days postpartum to AI	Calving date	Calving interval (days)
	03/28	04/08	04/13			
2434	-	2.06	10.22	89		
4063	-	11.02	9.09	64	01/28	343
4149	0.95	1.42	0.45	69	01/28	348
4337	0.40	0.21	0.75	90		
4464	-	3.66	0.27	58	01/30	339
4472	-	8.89	0.48	83	01/31	331
4487	-	0.51	14.87	50	02/07	339
4489	-	7.45	13.19	50	01/30	331
5089	2.10	1.18	0.66	74	01/23	347
6005	-	9.76	10.00	84		
6007	-	11.74	1.98	95		
6009	3.15	0.18	0.83	79		
6017	-	0.25	5.69	88		
6424	-	5.84	0.23	42	01/31	324
6492	0.61	0.37	0.13	39		
7300	-	1.40	7.26	101		
7381	-	9.44	0.18	70		
8024	-	1.11	6.93	87		
8464	-	0.05	3.19	87		
8476	-	5.06	7.34	94	02/03	379
8480	-	4.27	0.93	75		
8486	-	0.97	6.26	59		
8490	-	2.18	0.24	57		
9029	-	2.75	6.45	63	01/25	370

TABLE A5 - CALVING DATA AND SERUM PROGESTERONE CONCENTRATIONS ([P_4]) FOR CONTROL COWS (NO $PGF_{2\alpha}$) MAINTAINED AT DSAC, SIMPSON, IL.

Cow ID #	[P4] (ng/ml) date of sampling			Days postpartum to AI	Calving date	Calving interval (days)
	03/28	04/08	04/13			
9127	0.56	0.31	0.23	58		
9131	0.30	1.02	0.60	65		
9161	0.94	1.05	0.05	63		
9161	0.56	1.14	1.35	52		
9488	-	6.07	10.54	90		

TABLE A6 - CALVING DATA AND SERUM PROGESTERONE CONCENTRATION ([P_4]) FOR TREATED COWS ($PGF_{2\alpha}$) MAINTAINED AT DSAC, SIMPSON, IL.

Cow ID #	[P4] (ng/ml) date of sampling			Days postpartum to AI	Calving date	Calving interval (days)
	03/27	04/07	04/12			
0514	-	0.23	3.87	100		
0515	-	9.24	0.01	84		
0537	-	8.69	0.84	76		
0544	-	8.07	3.64	94		
0559	-	0.57	3.64	97		
0580	-	0.24	3.63	105		
0600	-	6.56	0.64	105		
0615	-	3.87	0.77	108	02/03	394
0622	-	11.63	0.45	104		
0644	-	3.71	4.20	61		
0999	0.69	0.17	0.14	58	01/30	340
1141	-	3.47	0.21	49		
2399	-	0.56	3.46	38		
4138	-	6.27	1.31	66	01/31	349
4483	-	0.01	3.56	86		
4495	0.61	1.17	0.19	51	02/01	335
5006	5.83	1.43	0.09	74		
5106	-	0.77	7.64	53		
5197	-	12.57	0.10	63	01/30	345
5233	-	11.04	0.69	68		
5488	-	3.61	1.02	49		
5499	0.67	0.01	0.66	46	02/05	334
6105	-	5.59	0.47	46		

TABLE A6 - CALVING DATA AND SERUM PROGESTERONE CONCENTRATION ([P_4]) FOR TREATED COWS ($PGF_{2\alpha}$) MAINTAINED AT DSAC, SIMPSON, IL.

Cow ID #	[P4] (ng/ml) date of sampling			Days postpartum to AI	Calving	
	03/27	04/07	04/12		date	interval (days)
6412	-	1.01	6.14	34		
7004	0.73	0.81	0.85	64		
7007	0.73	0.48	0.51	44		
7092	-	4.31	0.60	62		
7097	-	6.01	0.34	85		
7284	-	10.31	0.32	90		
7286	0.63	0.35	0.76	64	02/08	355
7288	-	7.17	0.70	-		
7295	-	0.19	1.96	62		
8006	-	8.69	0.01	-		
8032	5.16	0.18	0.04	-	01/24	-
8041	-	2.86	0.62	47	01/26	325
8051	4.67	0.01	1.07	97	02/01	381
8101	0.47	0.01	0.07	47		
8138	0.50	0.36	0.75	35		
8363	0.77	0.51	0.42	61		
8391	-	1.68	1.40	63		
8469	-	6.62	0.65	92		
8477	1.36	0.36	0.01	40		
9022	0.34	0.04	0.36	77		
9030	-	2.24	12.39	84		
9055	0.35	0.85	0.36	90		
9073	-	0.66	6.25	90	02/03	377
9098	-	3.10	2.92	95	02/03	381
9105	-	9.19	0.80	88		
9212	0.14	0.25	0.30	47		

TABLE A6 - CALVING DATA AND SERUM PROGESTERONE CONCENTRATION ([P_4]) FOR TREATED COWS ($PGF_{2\alpha}$) MAINTAINED AT DSAC, SIMPSON, IL.

Cow ID #	[P4] (ng/ml) date of sampling			Days postpartum to AI	Calving date	Calving interval (days)
	03/28	04/08	04/13			
X231	-	1.05	5.12	73		
197	-	2.33	0.06	73		
0506	-	0.34	4.00	66		
0517	-	6.44	0.24	104		
0533	0.47	0.96	0.62	58		
0588	-	7.95	0.40	114		
0592	-	7.07	0.15	110		
0602	-	3.87	1.25	105		
0606	-	6.00	5.52	58	01/29	338
0617	-	3.40	0.66	61		
0647	-	10.14	0.55	79		
0649	0.24	0.12	0.04	103		
0774	-	12.28	0.12	72		
0996	-	0.09	3.10	80		
0998	-	9.87	1.35	69	02/04	355
1334	-	8.46	0.32	92		
2404	-	0.17	6.98	92		
2409	-	9.29	4.00	80		
3114	-	9.93	0.02	74		
4182	-	0.06	4.80	90		
4189	2.37	0.76	0.07	89	02/06	377
4284	-	6.80	0.98	37		
4454	1.27	0.48	1.04	91		
4455	-	0.30	8.84	50		
4467	-	0.15	11.57	44	02/04	330
5129	-	2.50	11.28	66	02/01	349

TABLE A6 - CALVING DATA AND SERUM PROGESTERONE CONCENTRATION ([P_4]) FOR TREATED COWS ($PGF_{2\alpha}$) MAINTAINED AT DSAC, SIMPSON, IL.

Cow ID #	[P4] (ng/ml) date of sampling			days postpartum to AI	Calving	
	03/28	04/08	04/13		date	interval (days)
5143	-	8.95	0.41	89		
6008	-	3.10	0.57	93		
6036	2.17	0.25	1.16	84		
6043	-	2.87	0.18	90		
6432	0.35	0.27	0.81	63	02/02	347
7013	-	1.75	1.19	89	02/03	374
7272	0.36	0.01	1.10	70		
7275	-	7.73	6.67	59		
7292	0.59	0.87	0.15	97	02/05	384
7297	-	5.13	0.22	93		
8470	-	6.03	1.15	94	02/01	377
8493	-	0.01	2.72	89	02/01	372
8496	-	0.13	5.33	88		
9013	0.09	0.03	0.08	88	01/30	369
9062	1.50	1.17	0.71	40		
9065	0.59	0.22	1.20	87		
9072	1.02	0.15	0.73	39		
9168	-	7.87	8.87	93	01/30	374
9180	1.14	0.94	0.84	51	01/31	333
9199	-	8.25	0.16	98		
9226	0.60	0.49	0.31	65		
9476	0.61	0.15	0.10	84		
22482	-	3.94	0.18	93		

TABLE A-7. AMOUNT OF NORGESTOMET (μg) DAILY RELEASED FROM SILICONE IMPLANTS LOADED WITH DIFFERENT AMOUNTS OF STEROID AND SUBMITTED TO A SIXTEEN DAY IN VITRO ASSAY[1].

Day	Amount of norgestomet released (μg)	
	Implant load	
	6 mg	8 mg
01st	407 ± 21.2	455 ± 21.6
02nd	418 ± 36.6	485 ± 18.5
03rd	365 ± 25.6	445 ± 16.3
04th	322 ± 4.4	374 ± 31.7
05th	295 ± 11.6	363 ± 15.1
06th	276 ± 5.6	379 ± 14.2
07th	243 ± 9.1	307 ± 25.3
08th	220 ± 5.0	308 ± 20.2
09th	227 ± 6.4	296 ± 13.9
10th	209 ± 8.6	286 ± 28.9
11th	194 ± 17.5	245 ± 11.8
12th	209 ± 11.8	238 ± 13.2
13th	166 ± 5.1	232 ± 4.7
14th	174 ± 6.9	232 ± 9.0
15th	146 ± 3.9	235 ± 21.6
16th	143 ± 9.2	225 ± 28.0

[1] Values are means (± standard deviation) of four samples.

FIGURE A-1. DAILY RELEASE OF NORGESTOMET (μg) FROM THE IMPLANTS.

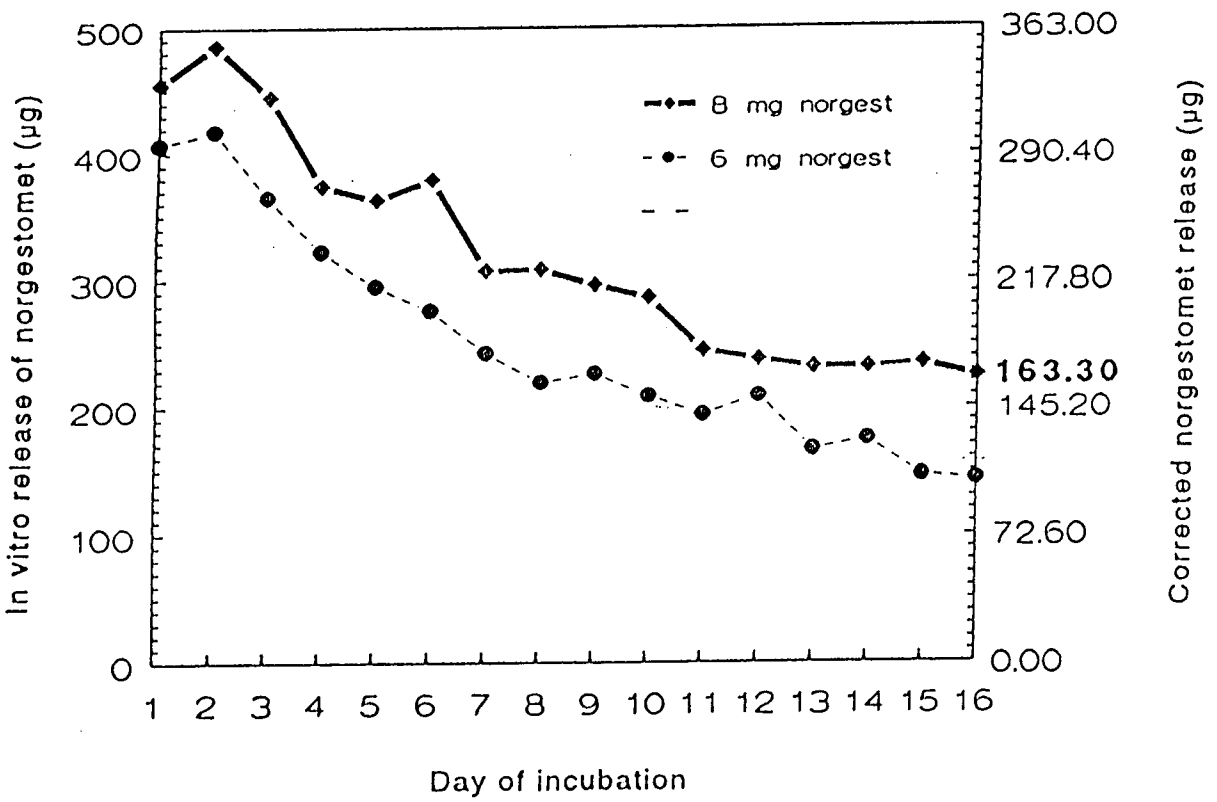

TABLE A-8. CUMULATIVE AMOUNT OF NORGESTOMET RELEASED (μg) AND DAILY RELEASE RATE (%) OBTAINED FROM SILICONE IMPLANTS LOADED WITH DIFFERENT AMOUNTS OF STEROID AND SUBMITTED TO A SIXTEEN DAY IN VITRO ASSAY.

Day	Implant load[1]			
	6 mg		8 mg	
	Mass (μg)	Rate[2] (%)	Mass (μg)	Rate[2] (%)
01st	407	6.78	455	5.68
02nd	825	7.47	940	6.43
03rd	1190	7.05	1385	6.30
04th	1512	7.17	1759	5.65
05th	1807	6.57	2122	5.81
06th	2083	6.58	2501	6.45
07th	2326	6.20	2808	5.58
08th	2546	5.99	3116	5.93
09th	2773	6.47	3412	6.06
10th	2982	6.48	3698	6.23
11th	3176	6.43	3943	5.69
12th	3385	7.40	4181	5.87
13th	3551	6.35	4413	6.07
14th	3725	7.10	4645	6.47
15th	3871	6.42	4880	7.00
16th	4014	6.72	5105	7.21
%Total		66.90		63.81

[1] Theoretical initial impregnated amount.

[2] Mass released on day n^{th}/mass left on day $(n-1)^{th}$.

TABLE A-9. CUMULATIVE AND DAILY RELEASE OF NORGESTOMET (μg) FROM SILICONE IMPLANTS LOADED WITH DIFFERENT AMOUNTS OF NORGESTOMET AND CORRECTED FOR KINETICS OF IN VIVO SECRETION[1].

Day	Type of implant[2]					
	6 mg[3]			8 mg[4]		
	Amount released (μg)			Amount released (μg)		
	Actual	cumul.[5]	Pred.[6]	Actual	Cumul.[5]	Pred.[6]
01	295	295	279	330	330	327
02	303	598	266	352	682	314
03	265	863	253	323	1005	302
04	234	1097	240	272	1227	289
05	214	1311	227	264	1541	276
06	200	1512	214	275	1816	263
07	176	1689	201	223	2039	251
08	160	1848	188	224	2263	238
09	164	2013	175	215	2478	225
10	152	2165	162	208	2686	212
11	141	2306	150	178	2864	200
12	152	2458	137	173	3037	187
13	121	2578	124	168	3205	174
14	126	2704	110	168	3373	162
15	106	2801	98	171	3544	149
16	104	2914	85	163	3707	136
%Tot		46.9			44.5	

[1] The correction factor was 0.726.
[2] Implants were designed to have either 6 mg or 8 mg norgestomet.
[3] Initial norgestomet content was 6.21 mg.
[4] Initial content of norgestomet was 8.33 mg.
[5] Cumulative mass released.
[6] Predicted daily release.

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